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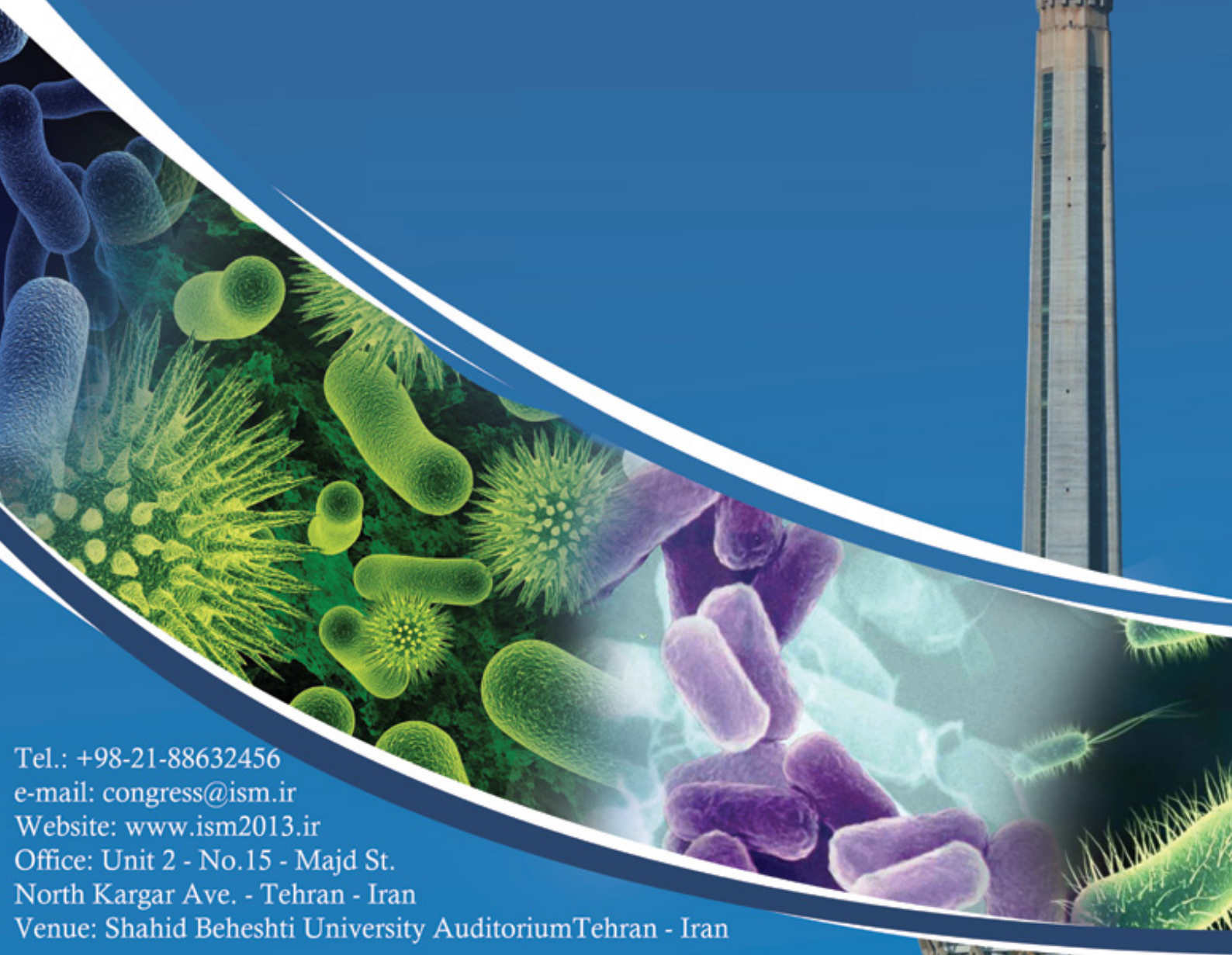
Abstract Book



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مرکز تحقیقات
علوم سلولی و مولکولی کردستان



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دانشگاه علوم پزشکی
و خدمات بهداشتی درمانی زنجان

دانشگاه علوم پزشکی و خدمات
بهداشتی درمانی کردستان

ارکان کنگره:

رئيس كنگره : دكتر محمدمهدي فيض آبادي
عضو هيئت علمي دانشگاه علوم پزشكي تهران

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عضو هيئت علمي دانشگاه علوم پزشكي اصفهان

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رئيس مركز تحقيقات ميكروبيولوژی كاربردی دانشگاه علوم پزشكی بقيه الله

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چهاردهمین کنگره میکروب شناسی ایران را در شرایطی برگزار می کنیم که مضایق مالی فعالیت های انجمن را با دشواری مواجه ساخته است. به همین دلیل برپایی همایش های سراسری و بینالمللی بدون داشتن پشتوانه های مالی کافی امری دست نیافتنی به نظر می آید. انجمن میکروبشناسی با آگاهی از این سختی ها اقدام به برنامه ریزی کنگره چهاردهم نمود. اگرچه انجمن در دوره جدید خود که از آبان ماه سال گذشته آغاز نمود فاقد منبع مالی بود، ذوق و انگیزه اعضای خود برای مشارکت در گردهماییهای علمی و فرهنگی را بزرگترین سرمایه و توشه راه خود دانسته و در این راه موفق به حمایت کامل اعضای خود در سراسر کشور گردید. استقبال گسترده اعضای انجمن موجب دلگرمی بیشتر برگزار کنندگان شده و تلاش ها برای برگزاری بهتر و با کیفیت آن فزونی گرفت. با پیشرفت کار، دانشگاههای متعدد و مراکز تحقیقاتی کشور به یاری کنگره شتافتند. برنامه های علمی کنگره مورد پشتیبانی وزارت بهداشت، درمان و آموزش پزشکی قرار گرفت و در نتیجه، امتیازات بازآموزی مناسب با برنامه ها به آن تعلق گرفت. علاوه بر حمایت وزارت بهداشت، کنگره مورد حمایت سازمان نظام دامپزشکی قرار گرفته و امتیازات باز آموزی به رشته های مرتبط با این حرفه نیز تخصیص یافت.

امسال چهارمین سال متوالی است که همکاری با انجمن های علمی خارج از کشور را در برپائی کنگره تجربه می نماییم و برای ارتقای روابط با مرکز علمی در جهان از اعضای خود در این راه استمداد می طلبیم.

همچنین برای استفاده بیشتر و بهتر از نظرات اعضای خود تقاضا مینماید اطلاعات مورد نیاز انجمن را در فرم مربوطه وارد نموده و پس از تکمیل آن را به کارشناس انجمن میکروبشناسی سرکار خانم احمدی که در محل غرفه انجمن حضور دارند تحویل فرمایند.

انجمن میکروب شناسی در امر داوری مقالات علمی دریافت شده به مجلات خود به همکاری اساتید و محققین کشور نیاز داشته تا در شرایط تحریم کیفیت انتشارات خود را ارتقا بخشیده و از این رهگذر بتواند زمینه همکاری و تعامل با همکاران را فراهم نماید.

اگرچه تلاشهای زیادی برای بهتر برگزارشدن کنگره انجام شده است، بدون شک اشکالاتی هم وجود خواهد داشت که در این خصوص نقدهای دلسوزانه اعضا را بجان خریداریم. بحث بیشتر در خصوص برنامههای علمی و کیفیت اجرای آنها را به دبیران محترم علمی و اجرایی سپرده و توفیق همکاران را از حضرت حق مسئلت می نمایم.

دکتر محمد مهدی فیض آبادی
رئیس انجمن علمی میکروب شناسی ایران و رئیس کنگره

پیام دبیر علمی کنگره:

با پیدایش تکنولوزیهای پیشرفته برای تشخیص و درمان بیماریهای عفونی وظائف و نقش میکروبیولوژیست‌ها اعم از دانشمندان، اساتید دانشگاهی، دانشجویان و کارشناسان آزمایشگاههای بالینی که بنوعی با آزمایشها و تستهای میکروبیولوژیک سر و کار دارند نیز بشدت در سالهای پیش روی دستخوش تحول خواهد شد. در حقیقت در سالهای اخیر ما شاهد یک دوره پویا و هیجان انگیزی در عرصه میکروبیولوژی در زمینه‌های مختلف بالینی، غذایی، دارویی، صنعتی و اکولوژیک بوده‌ایم. در این یکی دو دهه اخیر نه تنها بسیاری از روشهای میکروبیولوژیک دستخوش تغییر شده‌اند، بلکه انبوهی از لوازم و تجهیزات جدید و پیچیده هر روز وارد بازار شده و دست اندرکاران سیستمهای پزشکی و آزمایشگاهی و از جمله میکروبیولوژیک را در انتخاب آنها و جایگزین نمودن روشها و تجهیزات قدیمی دچار سر در گمی نموده است.

نقش دانشگاهها و مراکز آموزش عالی که در شرایط فعلی کشور ما با همه کمبودها و کاستی‌ها عهده‌دار تربیت نیروهای کارشناسی، آموزشی و پژوهشی در زمینه‌های مختلف میکروبشناسی هستند فوق‌العاده حائز اهمیت است. نگرش و نحوه عملکرد نیروهای جدید بشدت تحت تاثیر آموزه‌هایی است که در دوران تحصیلات عالی دیده‌اند. اما سئوال اصلی این است که در دنیای پیچیده میکروبیولوژی امروز دنیا ما در کجا ایستاده‌ایم. چه سهمی در تولید علم میکروبشناسی داریم. روشهای آموزشی و پژوهشی ما در زمینه میکروبشناسی بالینی چقدر پاسخ دهنده بیماریهای عفونی کلاسیک و نوپدید بالینی مردم ماست؟ علوم میکروبیولوژی در زمینه‌های صنعتی در این بین چقدر در تولید ناخالص ملی و ثروت ملی سهم دارند؟ دانش میکروبیولوژی غذایی در کشور ما چقدر توسعه یافته و در بهبود مواد غذایی از مزرعه تا سفره هموطنان چه نقشی ایفاء می نماید؟ دانش و پژوهشهای ما در زمینه میکروبیولوژی اکولوژیک چقدر در کاهش آلاینده‌های محیطی نقش داشته است؟.

برای پاسخ به این سئوالات منابع مختلفی باید جستجو شوند. اما کنگره میکروبشناسی که امسال چهاردهمین نشست خود را بر گزار می نماید هم یکی از این منابع است. نگاهی به خلاصه مقالات، ترکیب شرکت کنندگان، دانشگاهها و پوسترها و سخنرانیها و مقایسه آنها با دوره‌ها و کنگره‌های قبلی می‌تواند آینه تمام قد وضعیت امروز میکروبیولوژی کشور ما باشد.

کنگره امسال که همانند دوره‌های قبل به همت انجمن علمی میکروبشناسی ایران برگزار می‌شود، در شرایط بغرنجی برنامه‌ریزی شد. همه چیز در سال جاری دستخوش تغییر بود از نظام سیاسی کشور تا مدیریت دانشگاهها و از همه مهمتر وضعیت نه چندان مطلوب اقتصادی کشور. پیدا نمودن تامین‌کنندگان مالی یا اسپانسرها و دانشگاه مجری بزرگترین مشکل پیش روی کنگره بود. هیئت مدیره انجمن در برگزاری کنگره در این شرایط بسیار تردید داشت. به هر روی تصمیم گرفته شد که کنگره 92 در تهران و توسط خود انجمن و علارغم فقدان حمایت جدی هیچ دانشگاه و موسسه‌ای برگزار شود. در فاصله زمانی کوتاه و بدون اینکه خبر رسانی مطلوبی صورت گیرد، مقدمات کار صورت گرفت. استقبال کم نظیر و ارسال حدود 1200 خلاصه مقاله از سوی اساتید، دانشجویان، پژوهشگران و کارشناسان از اقصی نقاط کشور انجمن را در وضعیت غیر قابل بازگشتی قرار داد. کنگره با دعوت از اساتید بین المللی برجسته وجه بین المللی گرفت و پس از بررسی دقیق خلاصه مقالات و تعامل استثنایی با شرکت کنندگان که در قالب صدها رایانامه و پیامک و تلفن شکل گرفت امروز شاهد برگزاری کنگره 92 هستیم. امید است این کنگره نقطه عطفی در تاریخ انجمن و برگزاری کنگره‌ها باشد و بتواند محیط صمیمانه و مطلوبی را در اختیار شرکت‌کنندگان برای آشنایی با هم و تعامل علمی و دانشگاهی فراهم نماید.

پیشکسوتان علم میکروب شناسی

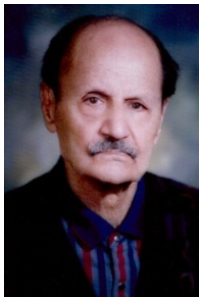
دکتر مهد خت پورمنصور

متولد تهران

دکتر مهدخت پورمنصور پس از اتمام دوره ابتدایی و متوسطه در تهران وارد دانشکده پزشکی دانشگاه تهران شد و بلافاصله بعد از اتمام دوره پزشکی در سال 1338 به عنوان دستیار و سپس رئیس آزمایشگاه بخش میکروب شناسی انستیتو پاستور ایران بخدمت مشغول گردیده است.

در سال 1350 به سمت رئیس بخش ب.ث.ژ منصوب و با حمایت معنوی و علمی شادروان دکتر مهدی قدسی بنیان گذار واکسن ب.ث.ژ در ایران ورئیس اسبق انستیتو به ساخت واکسن ب.ث.ژ اقدام نموده است. در سال 1363 به سمت رئیس بخش میکروبشناسی و مدیر گروه میکروبشناسی برگزیده و در سال 1370 مشاور انستیتو پاستور شده است. در سال 1373 بازنشسته و تا سال 1383 بعنوان مدیر پژوهش انستیتو پاستور انجام وظیفه نموده است.

در فاصله دوران خدمت موفق به دریافت تخصص میکروبیولوژی از فرانسه (سال 1347) و دکترای علوم آزمایشگاهی از دانشگاه تهران گردیده است. ایشان طی مدت خدمت مجری طرحهای تحقیقاتی و مقالات علمی متعدد بوده است.

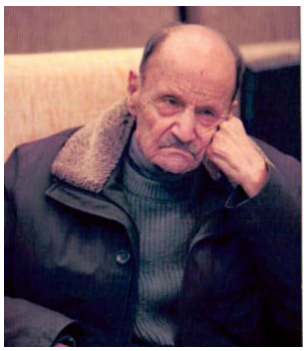


استاد احمد شیمی

پیشکسوت در تدریس میکروب شناسی به نسل های متعدد،

افتخار جامعه میکروب شناسی ایران

استاد فرهيخته جناب آقاي دكتر احمد شيمي پيشكسوت فرزانه، چهره ماندگار علمي كشور و بنيان گذار بخش بيماري هاي طيور دانشكده دامپزشكي دانشگاه تهران از ذخاير و گنجينه هاي گرانبهاي ميهن عزيزمان هستند . ايشان در سال 1294 در شهرستان بروجرد و در خانواده اي كارمند متولد شدند. فاميل ايشان معرف خانواده شان است. جد پدري ايشان معلم شيمي دارالفنون بودند و يكي از فرزندان جدشان نيز معلم شيمي دارالفنون بودند و اين دليل انتخاب فاميلي شيمي براي اين خانواده بوده است. دوره دبستان را در شيراز و دوره دبيرستان را در تهران گذراندند. در سال 1314 در دانشكده دامپزشكي تهران مشغول به تحصيل شدند و در سال 1318 با كسب رتبه اول از دانشكده فارغ التحصيل شدند و پس از ارائه پايان نامه اي درباره گسترش بيماري سورا در شتران ايراني به اخذ درجه دكتري دامپزشكي نائل گرديدند.



دكتر جوان شيمی ابتدا در اداره دامپزشكي خراسان و بعد در موسسه واكسن و سرم سازي رازي مشغول به خدمت شدند.در سال 1324 به دانشگاه تهران منتقل و به عنوان معاون آزمايشگاه ميكروب شناسي و مسئول تدريس درس عملي ميكروب شناسي منصوب شدند. در سال 1355 براي استفاده از بورسيه تحصيلي به دانشكده دامپزشكي دانشگاه كرنل در شهر ايتاكا در ايالت نيويورك معرفي گرديدند. پس از بازگشت به ايران آزمايشگاه ويروس شناسي و بخش طيور را پايه گذاري كردند و روش هاي قديمي ميكروب شناسي را به روش هاي رايج آن زمان در آمريكا و ساير كشور هاي پيشرفته تغيير دادند. علاوه بر آمريكا بورس هايي براي سوئد و انگليس گرفتند و تحصيلاتشان را در آن كشورها ادامه دادند. ايشان در سال 1345 با ارائه كتب و مقالات علمي متعدد به مرتبه استادي مفتخر و در سال 1359 پس از 35 سال خدمت دانشگاهي بازنشسته شدند. مدتي پس از بازنشستگي از دانشگاه تهران براي تدريس در دانشكده علوم و سپس دانشكده دامپزشكي دانشگاه آزاد اسلامي كرج دعوت شدند و تا 89 سالگي در آن دانشگاه مشغول به خدمت بودند.

تاليف و ترجمه كتاب هاي مختلف و مقالات و پژوهش هاي متعدد در حوزه هاي بيماري هاي باكتريايي و ويروسي، بيماري هاي طيور، ايمني شناسي دامپزشكي و ترجمه كتاب دامپزشكي بلاد و هندرسون نشان دهنده جامع بودن و اشراف اين بزرگوار در زمينه شاخه هاي مختلف علوم ميكروبيولوژي است.

استاد شيمی همچنين موفق به اخذ لوح تقدير و سپاس از بخش هاي مختلف گرديده اند كه از آن جمله مي توان به انتخاب ايشان به عنوان چهره ماندگار در رشته ميكروبشناسي در سال 1384 اشاره كرد.

تعداد قابل توجهي از چهره هاي پيشكسوت و ماندگار كشور تعليمات ميكروبشناسي و بيماري هاي عفوني دام و طيور را از استاد شيمي فرا گرفته اند. به نقل از شاگردان استاد شيمي، ايشان در مقام شامخ استادي با علاقه و محبت پدرانه، مناعت طبع و حس انسان دوستي راهنماي جوانان در جهت ارتقاي علمي و اخلاقي بوده اند.

استاد شيمي معتقدند هركس هر چيز را دوست داشته باشد به آن مي رسد و اين چيزي است كه ايشان در عمل با عشق به تعليم و پژوهش و كسب موفقيت در اين زمينه به گفتار خود صحه نهادند.

Abstracts

O1: **The Effect of Silver Nanoparticles on Experimental Wounds Contaminated with Pseudomonas aeruginosain in Mice**

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Background and Aim: One of the usual causes of nosocomial infections is wound site infection. Patients with these infections suffer of pain, loss of functional ability, decreased quality of life and may die. Wound infections intricate by pathogen bacteria. The microorganisms have been usual noted as the cause of delayed wound healing. The most common pathogen causing these infections is Pseudomonas aeruginosa. Nanoparticles are defined as particulate dispersions or solid particles with a size in the range of 10-100 nm. The alteration from microparticles to nanoparticles includes an increase in relation to the surface area, among other changes in properties. Silver nanoparticles (AgNPs) show both unique physicochemical properties (high ratio of surface area to mass) and ample antibacterial activities, which confer to them a major advantage for the development of alternative products against, for example, multidrug resistant microorganisms. Antibacterial nanoparticles (NPs) compared to traditional antibiotics, when offer many distinctive advantages in reducing acute toxicity, overcoming resistance, and lowering cost. AgNPs are attractive because they are non-toxic to the human body at low concentrations and have broad spectrum antibacterial actions.

Methods: Twenty Albino male mice were randomly divided into four equal groups (n=5). All mice were anesthetized with 250 µl doses of a Ketamine–Xylazine–saline cocktail (ratio 4: 1: 35) consisting of Ketamine100mg/kg and Xylazine 5 mg/kg, administered intra peritoneally. The hairs of mice were shaved, the exposed skin area were cleaned with 70% ethanol, and full-thickness skin wounds (3 mm in diameter) were created on the dorsal middle line of mouse using sterile biopsy punch equipment. 10 µl of the bacterial suspension (106CFU/ml) was added to each wound bed immediately after wound surgery. Animals groups include (AgNPs, tetracycline, AgNPs along with tetracycline and normal saline as control). In all groups treatments applied topically in the wound bed: 10 µl of AgNPs (0.04 mg/cm2) in AgNPs group, tetracycline (8 mg/kg) in tetracycline group, both of AgNPs (0.02 mg/cm2) and tetracycline (4 mg/kg) in AgNPs along with tetracycline group (half of normal dose) and saline normal in control group. Wound healing was monitored by taking digital photographs on days 0, 4, 8 and 12 post wounding. To determine healing efficiency, the residual wound size was measured from the unclosed wound area after on days 0, 4, 8, and 12 using Digital Adobe Photo Shop Software Histogram Analysis.

Results: Wound size was expressed as the percentage of the wound area determined on every post-wounding day, compared with the original wound area.The results show that the wound macroscopic healing significantly taken in treatment groups on day 12 (almost 100%), but only 79 percent area of the wound surface was closed in the control group.

Conclusion: Application of the dressings showed significant improvement in wound healing macroscopic rate. The AgNPs and tetracycline have a synergistic effect together.

Keywords: Experimental infection, Wound Size, Pseudomonas aeroginosa, Silver nanoparticles

O2: **Genetic diversity of Mycobacterium tuberculosis isolates in Iraq**

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Background and Aim: very limited data are available on genotypes of Mycobacterium tuberculosis isolates circulating in Iraq. In this study, we used Spoligotyping and MIRU-VNTR (15 locus) to explore the diversity of strain lineages and trace possible clustering in MTB isolates

Methods: a total of 134 non-replication M. tuberculosis clinical isolates recovered from 134 consecutive TB patients attending the National Reference Laboratory (NRL) in Baghdad during 2011. Data of susceptibility testing to four major antituberculous drugs using agar proportion method on Lowenstein-Jensen medium are available for 69 isolates. All isolates were typed by using spoligotyping and MIRU-VNTR (15 loci). Allele-specific PCR systems were used to detect specific SNPs known to confer drug resistance to rifampicin and isoniazid.

Results: At the lineage level, spoligotyping revealed that 92.6% of the isolates attributed to 4 lineages: CAS (42.5%), ill-defined T (29.9%), H (14.2%) and MANU (6%). On the basis of spoligotypes, 111 isolates were grouped into 16 clusters and the remaining 23 isolates were unique types, resulting in 39 distinct spoligotypes and a clustering rate of 70.9%. The SIT1144/T1 was the predominant (n=20, 14.93%). Before this study, the International database (SITVIT2) was containing only 4 isolates belong to this SIT (USA n=2, Venezuela n=1, Greece n=1). With MIRU-VNTR (15 locus), 30 isolates were grouped within 8 clusters and the remaining 92 isolates were unique types, resulting in 100 mirutypes and clustering rate of 18.03%. The minimum spanning tree (MST) showed a better resolution than spoligotyping alone. However, the highest discriminatory power was provided by spoligotyping and MIRUs together. On 15 locus MIRU-VNTR analysis, 14 loci were highly to moderately discriminate (h> 0.6, and h> 0.3, respectively), and, thus, could be used to discriminate the M. tuberculosis strains in Iraq. In addition, the mean of the allelic diversity of the 15 loci was high (0.65).

Conclusion: The molecular tools used in this study demonstrated several intersting results, several strains are shown to be successsfuly transmitted within the Iraqi people.

Keywords: Mycobacterium tuberculosis, Iraq, Spoligotyping, MIRU-VNTR

O3: **Detection of Bordetella parapertussis by Real-Time PCR: A population-based study in Iran**

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Background and Aim: Neglected parapertussis pathogen causes whooping cough-like disease is mostly milder and shorter than classic pertussis. The assumption of 20 to 30 percent of parapertussis incidence from whooping cough suspected patients is more than anticipated (< 5%). This is the first report of parapertussis prevalence and relatedness within Iranian patients.

Methods: The study was conducted for 1908 clinical samples and also was confirmed with culture and real-time PCR which determine 11 cases (0.57%) with PCR and 4 cases (0.20%) with culture. From age distribution point of view, parapertussis patients with mean age of 6.30 years represent a noteworthy difference ($P < 0.0001$) in comparison with the total sample. The genetic relatedness of the four B. parapertussis strains was analyzed by pulsed-field gel electrophoresis and two distinctive profiles were observed.

Results: This study gives a coherent assumption about B. parapertussis occurrence that revealed the incidence of 1.92 cases of confirmed parapertussis per 1000 person-years. In our samples, only 0.57% of all suspected cases infected by B. parapertussis represent a rarity of B. parapertussis in the population.

Conclusion: Finally this study reminds the necessity of continuous monitoring based on precise detection of parapertussis beside the pertussis and ongoing preventive measures for both organisms.

Keywords: Bordetella parapertussis, Real-Time PCR, Pulsed-field gel electrophoresis, Iran

O4: **Molecular analysis of Protein D in Haemophilus influenzae SPP in clinical isolates**

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Background and Aim: Haemophilus influenzae serotype b (Hib) is a major cause of bacterial meningitis and other invasive infections among children younger than 4 years. Since vaccination against Hib, other serotypes and nontypeable strains has emerged as significant cause of H. influenzae associated diseases. Amongst the H. influenza antigens, a highly conserved 42 kDa surface lipoprotein, Protein D is considered as a vaccine candidate. We aimed to determine the presence of hdp gene in clinical isolates of H. influenza and to assess the DNA polymorphism of the structural gene (hpd) encoding protein D.

Methods: Twenty isolates of H. influenzae were recovered from different clinical specimens of patients refereed to Milad and Imam Khomeini hospitals in Tehran. The bacteria were grown on Chocolate agar overnight at 37°C under 5% CO2 and were identified as H. influenzae using biochemical tests such as indole, urease, and ornithine decarboxylase. Following DNA extraction, the identity of isolates was confirmed via omp6 gene PCR. The hpd gene was amplified through PCR using gene-specific primers and the amplicons were digested with EcoR1. For five isolates, the amplicon of hpd gene were sequenced and the sequences were aligned with sequences of GenBank. Following this analysis, sequences were submitted to the EMBL accession numbers KC608167 , KC608168 , KC608169 , KC608170 , 1608499 , respectively.

Results: The 351bp amplicons of omp6 gene were obtained for 20 isolates and accordingly the identification of isolates was confirmed. Furthermore, an 1095-bp amplicon were observed for each isolate following hpd gene PCR. EcoRI restriction fragment length polymorphism (RFLP) patterns was detected in clinical isolates which were completely different from other GenBank records. Nineteen of 20 isolates had identical RFLP pattern. We found nucleotide substitutions in hpd gene sequences and most of nucleotide sequences produce nonsynonymous mutations. The nucleotide sequences and the deduced amino acids sequences for protein D in clinical isolates were highly conserved(>95 similarity).However, the few amino acid differences between the five isolates were located largely near the N and C-terminal ends.

Conclusion: We demonstrated that all examined clinical isolates of H. influenzae possessed the hdp gene and only a limited variation were found within the gene. Due to the presence of hdp gene in all clinical isolates and the conserved amino acids sequences of protein D, Protein D need to be characterized and considered as a novel vaccine candidate against all types of H. influenzae.

Keywords: Haemophilus influenzae NTHi, Protein D, Haemophilus influenzae type b,PCR, EcoR1, FLP

O5: **Construction and assessment of immune responses of a new vaccine candidate against urinary tract infection**

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Background and Aim: Urinary tract infection (UTI) caused by Uropathogenic Escherichia coli (UPEC) is one of the most common infections in the world. Type1 pili by having the adhesin FimH is the important virulence factor of UPEC strains. Despite extensive efforts, a vaccine that confers protection against UTI in human is currently lacking and thus we need to test new target antigens to develop an ideal vaccine against UTI. In this study, we constructed recombinant fusion fimH.fliC of UPEC as a novel vaccine candidate against UTI and then the adjuvant property of FliC was evaluated in mice immunized with purified FimH protein admixed or genetically linked as fusion protein. Finally, efficacy of the immune responses was evaluated for protection of the bladder and kidney of challenged immunized mice.

Methods: The fusion fimH.fliC was constructed by overlap PCR and the gene expressed in pET28a vector. Endotoxin level of the proteins was determined by the LAL test and the TLR5 bioactivity of the fusion tested by IL-8 induction in HT29 cell. Mice were subcutaneously immunized three times with 50 µg of proteins (FimH, FliC or fusion). Total IgG, IgG isotypes (IgG1, IgG2a), IgM and IFN-γ, IL-4 and IL-17 responses of immunized mice were measured by ELISA. Finally, mice were inoculated transurethrally with 108 CFU of bacterial suspension and the bladder and kidneys were cultured on LB agar to determine the CFU/ml of the tissues.

Results: Mice immunized with the fused FimH.FliC protein induced significantly higher humoral (Total IgG, IgG1 and IgG2a) and cellular (IFN-?, IL-4 and IL-17) immune responses than with FimH alone or FimH admixed with FliC. Our results showed that based on the IgG1/ IgG2a ratios, FliC directed the anti-FimH responses preferentially towards Th2. The FimH.FliC fusion combination gave the best results in protection of bladder and kidneys colonization, compared to the control group.

Conclusion: To date, there has been limited success in developing an efficacious vaccine against UTI. The flagellin (FliC) as adjuvant activates the host immune system via interaction with TLR5. We hypothesized that genetically linking of FliC as adjuvant and FimH as antigen can significantly increase the immunogenicity of FimH. Among UPEC antigenic proteins, FimH was reported to be highly immunogenic and induces protective immunity against UTI in mice and primate models. Our results propose new promising vaccine candidate based on the adjuvant properties of FliC against UTI caused by UPEC strains.

Keywords: Urinary tract infection, Uropathogenic Escherichia coli, vaccine, fusion, adjuvant, flagellin

O6: **Comparison of the effects of Tio2 nanoparticles on pathogenic bacteria(MDR) prokaryote model and Wistar rats as a eukaryote model**

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Background and Aim: Because of its excellent optical performance and electrical properties, TiO2 has a wide range of applications in many fields. It is often considered to be physiologically inert to humans. However, some recent studies have reported that nano TiO2 may enerate potential harm to the environment and humans. In this paper the in vitro and in vivo toxicity of nano TiO2 particles on bactrria and rat was investigated.

Methods: A total of 200 clinical samples in 4 hospital of Isfahan city was studied during 6 months. The MDR assay was performed by disk diffusion method. Minimum inhibitory concentration (MIC) values . A total of 32 healthy male Wistar rats were randomly divided into four groups: control group (treated with /5 cc normal saline) and three experimental groups. Group 1, 2 and 3 received. /5 cc of solution containing 10,100,300 ppm TiO2 (10-15 nm) via IP injection for 7 successive days, respectively. The control group was treated with same procedure. The effects of nanoparticles TiO2 on serum biochemical levels serum glutamate oxaloacetat transaminase (SGOT) and serum glutamate pyrvate transaminase (SGPT) were evaluated at various time points (2, 7 and 14 days). After 14 days, the tissue of liver and lung was collected and investigated

Results: Of the 200 patients studied, 144 (72.0%) had gram-negative bacilli containing ESBL and 56 (28.0%) had gram-negative bacilli without ESBL ,but only MDR and the most prevalent bacteria was identified as Klebsiella pneumonia, with especially strong resistance to cefotaxime. All of these bacteria were sensitive to the Au nanoparticle solution with density of 100 ppm, but the 10-15nm size .In this study indicated that after 2 days, mean level of SGOT in groups 1 and 3(Tio210ppm, Tio2300) significantly increased in comparison control group (0.002 p.v=0.009) respectively. Seven and 14 days after the injection of Tio210ppm, Tio2300ppm, and the liver damage returned. Also, level of SGPT in groups 3 significantly increased in comparison group1 and group 2 (Tio210ppm, Tio2100ppm)(p.v=0.027, p.v<0.0001).In this study, higher doses of Tio2) 300ppm) induce gravely toxicological effects on enzymes hepatic. No significant effects were noted for SGPT after consumption of three doses of nanogold.Histopathological examination of lung in Group 1: destruction of air sac and their collapse-destruction of air sac, wasting of spaces air sac and hyperemia of vessels (group2)-increasing of diameter air sac and fibrosis of spaces connective tissue (group 3) indicated. Every three groups induce heavy injuries on lung.

Conclusion: The results seem to indicate a direct correlation between Tio2 nanoparticle solution concentration and the diameter of growth zone for pathogenic bacteria(MDR). Assays in our study were in vitro; if use of Tio2nanoparticles in vivo proves to be with adverse effects, it could be a valuable alternative to antibiotics

Keywords: Gram-negative bacilli , MDR, Tio2 nanoparticles, Wistar rat, Toxicity

O7: **Analysis of Quorum sensing related genes in virulence of different genotypes of Pseudomonas aeroginosa isolated from keratitis and conjunctivities.**

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Background and Aim: Pseudomonas aeruginosa is a Gram-negative, opportunistic pathogen implicated in sight-threatening ocular infectious diseases such as keratitis. Pseudomonas keratitis is a serious ocular infection that can lead to corneal scarring and severe visual disability if aggressive and appropriate therapy is not promptly initiated. Emergence of resistance to fluoroquinolones which are widely used for eye infections has complicated the treatment of these infections. Quorum sensing is the system which controls many of virulence factors in the Pseudomonas aeruginosa and loss of this system impairs the virulence of the bacterial isolate. On the other hand bacterial control does not necessarily require bactericidal activity; it may be sufficient to target genetic pathways that are essential for virulence or the infection process. Two quorum-sensing systems, the las and rhl systems have been described in P. aeruginosa. The las system controls the expression of virulence factors such as elastase and toxin A. Second quorum-sensing system, rhl regulate the production of haemolysin and pyocyanin, as well as elastase and alkaline protease. Thus, if the QS system of P. aeruginosa can be manipulated, it may be possible to control the virulence of and infection by this aggressive pathogen. In IRAN in spite of widespread use of contact lenses and emerging drug resistance there are not many studies regarding these bacteria and their virulence factors. Therefore in this study we are going to investigate about quorum sensing related genes in different genotypes of P. aeruginosa. These genes include lasI ،lasR ،aprA ،rhlI ،rhlR and rhlAB which involving in eye infections pathogenesis.

Methods: The samples were collected from patients having conjunctivitis or keratitis referring to Farabi Hospital in winter of 2010. After confirming the isolates as Pseudomonas aeruginosa the drug resistance of samples was assessed using disk diffusion method. Presence of proteases including elastase and alkhaline protease was investigated using Zymography method. The presence of quorum sensing related genes was analyzed using PCR method. Genetic analysis of the P. aeruginosa strains was performed by REP-PCR and the results were analysed using Free tree and Mega softwares.

Results: All strains were resistant to clindamicin, co-trimaxazol, vancomycin and nifuroxazide. On the other hand, all isolates were susceptible to amikacin, ciprofloxacin, ceftazidime and gentamicin. In Zymography isolates showed a three band pattern including 200, 150 and 50 KD bands which were related to protease IV, elastase and alkhaline protease activity. All isolates showed protease IV and elastase bands but only two isolates showed alkhaline protease activity. All of 60 clinical isolates harbored all of quorum sensing related genes including lasI ، lasR ،aprA ،rhlI ،rhlR and rhlAB. Genetic analysis of the P. aeruginosa strains by REP-PCR disclosed different patterns. Most of patients with P. aeruginosa keratitis harbored different genotypes with their unique pattern.

Conclusion: It could be conclude that quorum sensing related virulence factors play an important role in pathogenesis of P. aeruginosa in eye infections. Also our genotyping results showed that our isolates were not epidemiologically related to each other.

Keywords: pseudomonas aeruginosa, virulence factors, Quorum sensing, eye infection

O8: **Comparison of the RE and B1 gene for detection of Toxoplasma gondii infection in children with cancer**

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Background and Aim: Early, accurate and effective diagnosis of toxoplasmosis can make an important contribution to the prevention and control of disease, especially in people who are at risk. In this study, two commonly used genomic repeats of Toxoplasma gondii, RE (GenBank accession number AF146527) and B1, were compared to each other in nested-PCR assay.

Methods: Five hundred and thirty-five blood samples from children with leukemia were tested for the presence of T. gondii antibodies using enzyme immunoassays. One hundred and ten DNA samples of these patients (50 IgM+ IgG+, 10 IgM- IgG+ and 50 IgM-, IgG-) were analyzed by nested-PCR. The specificity of two nested PCR assays was determined using the DNA samples of other parasites and human chromosomal DNA.

Results: As a result, 82% (41/50) and 68% (34/50) of the IgM+, IgG+ samples were positive on duplicate RE and B1-nested PCR analyses, respectively. None of the 10 IgM-, IgG+ seropositive samples was detected positive after testing RE and B1-nested PCR assays in duplicate. One (2%) of the 50 seronegative samples was positive by duplicate RE-nested PCR but none of them were positive by duplicate B1-nested PCR. The detection limit of the RE-nested PCR assay was 640 fg of T. gondii DNA whereas this rate for B1-nested PCR was 5.12 pg of the DNA template. No cross-reactivity with the DNA of other parasites and human chromosomal DNA was found.

Conclusion: The results indicate that an RE-based nested PCR assay is more sensitive than B1 genomic target, of those tested, for detection of T. gondii. It is noteworthy that in comparison with B1-nested PCR, RE-nested PCR could detect the T. gondii DNA in seronegative samples too.

Keywords: RE, B1, Nested-PCR, Toxoplasma gondii, Children with cancer

O9: **Quasispecies Development of Helicobacter pylori Infection in Iranian Patients**

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Background and Aim: The human pathogen Helicobacter pylori (H. pylori) is associated with the development of a variety of gastroduodenal diseases. H. pylori strains that can exist within their hosts as pools of related genetic variants, referred to as quasispecies. Such a population of strains shares a common origin but have distinct genomic sequences as a result of genomic diversity through natural competence, high mutation and recombination capacity and variation in gene order or differences in their genomic contents. The aim of current study was to determine the development of quasispecies H. pylori infection among the Iranian patients by comparing their Multiplex-PCR genotyping and Random Amplified Polymorphic DNA (RAPD) patterns.

Methods: Gastric biopsies were taken from antrum of 98 patients subjected to endoscopy procedure for their gastric disorders in Taleghani Hospital. The biopsy specimens were cultured on Brucella agar supplemented with 7% sheep blood and antibiotics in 37 ºC at microaerophilic conditions for 3-10 days. Isolates were identified by conventional biochemical and molecular tests. Pure cultures of 6 single colonies were used for DNA extraction from each sample. A Multiplex-PCR method based on different genetic markers was designed for simultaneous screening of genotype diversities of the bacterial isolates for cagA, vacA (s1/s2), vacA (m1/m2), iceA1/A2 genes. RAPD pattern of each isolate was determined using primer pairs 1254 and 1283, separately. Genetic relatedness of the isolates in each patient was analyzed by comparing the RAPD patterns using Gelcompar II software. Existence of differences of ≤ 2 RAPD bands together with established allelic diversity of the studied virulence genes were considered as definitive criteria for detection of quasispecies development in each patient.

Results: Results obtained from this study showed that 39 out of the 98 studied patients (39.8%) were positive for H. pylori infection. We assessed a total of 232 single isolates from these patients (i.e. six isolates from each sample). Seven (17.9%) of the infected patients showed quasispecies development of H. pylori infection. Genetic diversity of strains with related RAPD patterns in each sample were determined on the basis of the following gene conversions: vacA m1: m2 (28.5%), cagA+: cagA- (42%), and iceA A1: A2 (28.5%).

Conclusion: H. pylori isolates obtained from individual hosts show considerable genomic diversity. According to current study, we hypothesize that genetic drift may be responsible cause of genetic variation among multiple H. pylori isolates in each patient. These findings proposed the quasispecies development of Helicobacter pylori infection in Iranian patients. Our results showed that application of multiplex-PCR genotyping combined with RAPD typing can be useful in detection of quasispecies infection in H. pylori infected patients.

Keywords: Helicobacter pylori, Quasispecies, Multiplex-PCR, RAPD

O10: **Study the potential of a new Brucella melitensis recombinant Dnak in conferring protection against brucellosis in Balb/c mice**

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Background and Aim: Brucella melitensis infection is still a major health problem for human and B. melitensis Rev.1, an attenuated smooth strain used to control B. melitensis infection but because of some problems a subunit vaccine that is protective against B. melitensis is desirable. In order to study the potential of a new Dnak protein for the development of a brucellosis subunit vaccine, immunogenicity and protective efficacy of recombinant Dnak from B. melitensis was evaluated in BALB/c mice.

Methods: Firstly, Dnak gene was cloned and expressed. Different mice were immunized by the intraperitoneal route with 30 µg of recombinant Dnak, PBS and Rev.1 vaccine. Then, LTT assay, Cytokine assay, specific IgG1, IgG2a assay and protection assay were done.

Results: The recombinant Dnak generated high IgG antibody levels with higher IgG2a than IgG1 titers. In addition, after adding rDnak to spleen cells of immunized mice in vitro, the splenocytes started to proliferate and produced significant amount of IFN-?, IL-12, IL-10 and IL-6. Therefore, Th1 response is probably induced against rDnak. The protective effect of rDnak was evaluated by administering rDnak in combination with adjuvant to mice that resulted in a significant degree of protection against B. melitensis infection compared to mice immunized with PBS($P<0.001$) but lower than Rev.1 induced protection.

Conclusion: In present study, rDnak induces partial protection and a Th1 response against B. melitensis infection showed that they are important in clearance of B.melitensis infection in host. Therefore, Dnak maybe a useful candidate for the development of subunit vaccine against brucellosis.

Keywords: Brucella, Recombinant, Vaccine, Protection, LTT, Cytokine

O11: **Studying the effect of quorum sensing on swarming of Aeromonas caviae**

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Background and Aim: Mesophilic Aeromonas are ubiquitous water-borne bacteria. In humans two major diseases associated with Aeromonas are gastroenteritis and wound infections. Flagella are among many virulence factors, which have been recognized for Aeromonas spp. and have multiple roles that contribute to pathogenesis. These bacteria possess two distinct flagellar systems; i) a polar flagellum for swimming in liquid and ii) lateral flagella for swarming over surfaces. Aeromonas lateral flagella, in addition to mediating swarming motility, appear to be adhesins, contribute to microcolony formation and efficient biofilm formation on surfaces, and possibly facilitate host cell invasion. It is, therefore, likely that the ability to express lateral flagella is a significant virulence determinant for the Aeromonas strains able to cause persistent and dysenteric infections in the gastrointestinal tract. Previous studies have suggested that quorum sensing (QS) is contributed to swarming. Quorum sensing is a kind of bacterial communication which can be conducted by small diffusible molecules which is a widespread phenomenon in many species. This form of communication can regulate the gene expression of lots of biological functions such as antibiotic production, biofilm formation and virulence. When a population is getting too large, QS regulation of swarming most likely allows optimal diffusion of bacterial cells to inhibit a single given niche. Recently QS has been considered as a good target for anti-microbial therapy as well. In many Aeromonas species quorum-sensing regulation is mediated by the production of the AhyI protein catalyzing the reaction for production of acyl-homoserine-lactones. So far, there is no available study on the effect of QS on swarming of motile Aeromonas. Therefore we aimed to investigate this effect by knocking out the cognate genes and observation of any probable phenotype changes in swarming

Methods: A. caviae strain Sch3N was used as a swarming positive Aeromonas strain . A ΔahyIR mutant strain of A. caviae sch3N was constructed by insertion of kanamycin (Km) cassette at the middle of the QS cognate genes in a way to produce a double mutation in ahyI and ahyR in a time. The mutant gene then cloned into a suicide plasmid and following the transformation of E.coli S17λpir, the recent plasmid was introduced to A. caviae Sch3N through conjugation. Double homologous recombinant strains of mutants were selected on Km/Nal plates and confirmed by PCR. The mutant strain was employed for investigation of the swarming on the specific swarming plats and phenotype changes was compared with the wild type. The experiment was repeated on different swarming plate.

Results: The obtained results showed that the ahyIR defective strain of A. caviae had had swarmed on a solid surface in a similar way of the wild type.

Conclusion: It is concluded that ahyI and ahyR genes involved in quorum sensing signaling doesn't plays a major role in control of the A.caviae swarming and may not affect the bacterial attachment to the epithelial cells. The effect of other QS signaling systems must be considered for further investigations.

Keywords: Aeromonas, swarming, quorum sensing

O12: **Improved cultivation conditions for maximum production of PRP by Haemophilus influenzae type b in Fed-batch Fermentation**

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Background and Aim: The production of the capsular polysaccharide, polyribosylribitolphosphate (PRP), from Haemophilus influenzae type b (Hib) is important for the production of effective conjugate vaccines. This work deals with the study of capsular polysaccharide biosynthesis by 2 local isolate of H influenzae b in shake flask and fed batch fermentation.

Methods: Several experiments were conducted to select the best strain, investigate the proper size inocolum and the influence of nutritional and environmental conditions for optimum polysaccharide production.

Results: Kinetic studies showed that the highest level of the macromolecule (approximately 188 105 mg/L) was obtained with initial cell concentration of around 20 mg/L. Increased concentrations of carbon source negatively affected the fermentation process whereas increasing the yeast extract concentration improved the polysaccharide synthesis.

Conclusion: The cyclic feed batch was a promising system allowing higher product concentration than the batch process. The polysaccharide recovered and partially purified was of high molecular weight.

Keywords: Haemophilus influenzae b, Capsular polysaccharide, PRP, fermentation,

O13: **Immunogenicity Comparison of Conjugate Vaccines of Alginate and Detoxified Lipopolysaccharide of Pseudomonas aeruginosa bound to Diphtheria Toxoid**

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Background and Aim: Treatment of Pseudomonas aeruginosa infections is greatly hampered by innate and acquired antibiotic resistance. Pure polysaccharides are poor immunogens and most of them are T-lymphocyte independent (TI) antigens. Polysaccharide-protein conjugates as vaccines could be a way to convert a TI to T-lymphocyte dependent (TD) antigen. The goal of this study is immunogenicity comparison of conjugates of P. aeruginosa alginate-diphtheria toxoid (ALG-DT) and P. aeruginosa detoxified lipopolysaccharide-diphtheria toxoid (D-LPS-DT) against P. aeruginosa infections in mouse model.

Methods: Alginate and LPS were purified from P. aeruginosa of strain PAO1. The resulting depolymerized alginate and detoxified LPS were covalently coupled to diphtheria toxoid (DT) as a carrier protein with adipic acid dihydrazide as a spacer molecule and carbodiimide as a linker. Tests for sterility, general safety of the vaccines and pyrogenicity testing were performed. 30 mice in two groups were immunized intraperitoneally on days 0, 14 and 28 with 10 µg of ALG-DT and D-LPS-DT. Alginate, LPS and protein antibodies level were determined by an enzyme-linked immunosorbent assay (ELISA).

Results: The conjugates were non-toxic for mice and non-pyrogenic at a dose of 10 µg/kg of body weight when intravenously administered to rabbits. Conjugates of ALG-DT and D-LPS-DT were shown to be safe and to elicit total IgG, IgM, IgA, IgG1, IgG2a, IgG2b and IgG3 antibodies in mice. Results of ELISA indicated that antibodies titer of ALG-DT is more than D-LPS-DT.

Conclusion: Immunization with ALG-DT showed significance increasing in all types of antibodies titers concentration in versus D-LPS-DT, which ALG-DT can be used as a vaccine candidate against P. aeruginosa infections.

Keywords: P. aeruginosa, LPS, Alginate, Conjugate Vaccine, DT

O14: **Identification of Bordetella pertussis by Real-Time PCR using IS481 and BP283 targets**

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Background and Aim: Introdiction: Rapid detection of whooping cough disease is very important in treatment and epidemiological studies. Molecular methods used for detection of B. pertussis in clinical swabs are more rapid than culture and biochemical methods. In order to diminish the false positive results both IS481 as a multiple copy sequence with high sensitivity and BP283 with a single copy number with high specificity are used for B. pertussis detection. In this study we aim to increase specificity of real-time PCR assay by use of BP283 target as a confirmative sequence.

Methods: Material and methods: For validation of real-time PCR of B. pertussis, a total of 698 nasopharyngeal specimens were cultivated in regan-lowe medium with and without cephalexin(40 µg/ml). Genomic DNA of the samples were extracted with DNA extraction kit (Roche). In this method we used two sets of primers named IS481 and BP283 to identification of B. pertussis genome in collected samples and also used primer amplifying glyceraldehyde-3-phosphate dehydrogenase (GAPDH) housekeeping gene for internal control (IC) to detect the presence of Taq DNA polymerase inhibitors.

Results: Results: Out of 698 samples 9 samples were culture positive and112 and 95 cases were detected as B. pertussis using IS481 and BP283, respectively. Ninety five cases were positive with both targets. In our results the IS481 had sensitivity and specificity of 100% and 85% and BP283 had sensitivity and specificity of 100% and 87.5%, respectively compared to culture.

Conclusion: Discussion: In this research 17 cases were false positive by using IS481 that means cross reaction of this target with other species. These results had similarity to other studies about two specific targets which used in this study. It is suggested simultaneously use of IS481 and BP283 with higher specificity to increase the assurance of the real-time PCR assay for molecular detection of B. pertussis. We can develop PCR method for specific detection of Bordetella spp. by using primers which enable discrimination of these species of Bordetella.

Keywords: Keyword: Bordetella pertussis, Real-Time PCR, IS481, BP283

O15: **Recombinant antibodies directed against surface proteins of Clostridium difficile**

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Background and Aim: Clostridium difficile is an anaerobic gram positive and spore forming bacteria of the genus clostridium, first discovered in 1935 and reported as antibiotice associated diarrhoea disease in the late 1970s that could lead to pseudomembranous colitis. Main virulence factors, toxin A and B, can be produced after adherence of bacteria cell to the intestinal epithelium. Analysis of bacterial adherence and immunological studies revealed that surface proteins have a pivotal role in colonisation and potentially can be considered as vaccine candidates. Based on analysis of the genome of C.difficile 630 and proteome analysis the surface proteins can be generally divided into 2 groups. First group could be those with copies of cell wall binding Pfam04122 such as Cwp84. Second group with different motifs including factors like flagellum. The aims of this study were to isolate recombinant antibodies against recombinan targets of Clostridium difficile via antibody phage display and assess them if they could use in inhibition function the targets and to confirm their location.

Methods: The genome sequence of Clostridium difficile 630 (NC-009089.1) was used to design all primers for amplification of the selected coding sequences. The pET-32 EK/LIC vector was used as the cloning and expression system. Colony PCR and DNA sequencing were used to confirmed the tranformants. The human single fold scFv libraries Tomlinson used for selecting scFv antibodies able to bind to the recombinant clostridial target proteins via phage display. Polyclonal and monoclonal phage ELISA was applied to select the best binders. The selected scFvs were characterised through different methods after preparing HB2151 clones of each and analysing of their CDR sequences.

Results: Expression and purification of 8 clostridial proteins were used (CspA, GroEL, FliC, FliD, putative sortase, Cwp66, and its amino and carboxy terminal regions) for antibody isolation. Phage display yielded a large panel of specific scFv antibodies that were expressed, purified and characterised.Reaction between the scFvs and their targets was checked e in ELISA and Western blotting suggesting the recognition of mostly linear epitopes. Strain specificity with good recognition of protein from C. difficile 630 was revealed of binding scFvs to SlpA with no reaction towards other riobtypes. The reaction of scFvs against flagella proteins determined an inhibition motility of bacteria. Some scFvs were tested in immunofluorescence microscopy. The positive results from these experiments showed that the reagents and the strategy pursued could be used to establish surface exposure of the targets and other components of the bacterial surface. Given the high specificity of the reagents, and in the case of Cwp66, the ability to isolate scFvs against defined regions of the protein, the strategy has the capacity to define the orientation of proteins in the bacterial surface.

Conclusion: Overall, expression of proteins from C. difficile in an E. coli host was generally successful and phage display provided a rapid, highly efficient method for the isolation of specific immunological reagents. These have the potential to explore the location, orientation and activity of proteins from the pathogen.

Keywords: Clostridium difficile, phage display, scFv, cloning and expression

O16: **Prevalence of Haarlem Family in Mycobacterium tuberculosis in world population: Systematic Review and Meta-Analysis**

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Background and Aim:: One of the most prevalent genotype of Mycobacterium tuberculosis in global population is Haarlem family that are associated with drug resistance The aim of this study was to determine the prevalence and distribution of Haarlem family in the world using meta-analysis based on systematic review of articles published.

Methods: All original articles published in literature database including PubMed, Science direct, Web of Science, Google Scholar, Biological abs, Iranmedex, and SID systematically reviewed prevalence of Beijing family. Data analyzed using meta-analysis with random effects models.

Results: Final analyses included 78 samples that have been selected from 2162 studies. Overall Haarlem family prevalence in world was estimated to be 8.6% (95% CI 8.3–8.9). Corresponding estimates by continent were Europe 13.8% (13.1–14.5), Asia 9.5% (9–10.1), America 6.8% (6•2–7.4) and Africa 4.2% (3.5–5).

Conclusion: According to the results, this genotype is prevalent in European countries. Effective control program is needed in world to control the spread of drug resistance strains specially Haarlem family.

Keywords: Haarlem, M. tuberculosis, Prevalence, Meta-analysis

O17: **Phylogenetic, immunological and Full Genomic Analysis of Iranian HIV Subtypes in Comparison with animal immunodeficiency viruses**

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Background and Aim: Feline Immunodeficiency Virus (FIV), Bovine Immunodeficiency Virus (BIV) and Simian Immunodeficiency Virus (SIV), are closely similar to the Human Immunodeficiency Virus (HIV) in genomic organization, morphology, physical and biochemical features and manifestation of disease. Although all of these viruses belong to Retroviridae family and Lentivirus subfamily, there is a significant difference between rate of similarity, antigenicity and genetic properties.

Methods: After basic study, the complete genome sequences of Iranian HIV virus isolates and SIV, FIV and BIV retrieved from databases. Then Iranian sequences were aligned and analyzed by using of wide array of software and online tools and compare with SIV, FIV and BIV. On the next step, the highly conserved and variable Region of genome was evaluated by revolutionary views .A query sequence submitted to a BLASTp search in HIV database and NCBI. Then the phylogenic tree was drawn with 3 methods: Neighbor-joining tree, Minimum- evolution tree, UPGMA tree and also overall mean distances were estimated. At end comparative antigenic and immunogenicity analysis was done on surface glycoproteins of HIV with other animal viruses

Results: Analysis of sequences was shown, from the whole number of Lentivirus subfamily, just 10 sequences are available in NCBI and other databanks. The result of alignment and evaluation was revealed significant similarity and homology between Iranian HIV Subtypes with SIV, FIV, and BIV respectively was %65, %50 and %47. Blast results was shown close resemblance between Iranian sequences with Afghanistan. Less comparative similarity was seen with subtypes of Kenya and West Africa genomic sequences. In the investigation of phylogenetic tree, SIV had the closet structural resemblance with HIV. Data was shown less similarity between FIV and BIV with HIV. One of the subtypes that was isolated from Tehran had high similarity to SIV. Antigenic and immunogenicity analysis of Human immunodeficiency virus with domestic animals was shown a relative conservation of epitopes in major core constituent proteins of surface antigens.

Conclusion: Prevalence of HIV disease in some areas such as Iran and African countries causes more attention to studies about this field. Phylogenetic studies provide a moderate base for discovering revolutionary relationship between different viruses, producing effective vaccines against HIV and other members of Lentivirus subfamily and finding an appropriate remedy to prevent HIV affliction.

Keywords: Human immunodeficiency virus(HIV), Simian immunodeficiency virus(SIV), Feline immunodeficiency virus(FIV), Bovine immunodeficiency virus, Genomic analysis, phylogenetic comparison

O18: **Resistance of nanobacteria isolated from urinary and kidney stones to broad-spectrum antibiotics**

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Background and Aim: Nanobacteria or calcifying nanoparticles (CNP) possesses unique properties such as small size (0.1 to 0.5 microns) and high resistance to heat and routine antimicrobial agents. These organisms are 100 times smaller than bacteria and protected by a shell of apatite, so they could be as candidate for emerging and progress of in vivo pathological calcification. In this study inhibitory effect of broad-spectrum antibiotics on growth of these new forms of life has been investigated.

Methods: Powdered urinary and kidney stones were demineralized with HCl and neutralized with appropriate buffers and became filtered. Finally suspension was incubated in DMEM medium with Fetal Bovine Serum (FBS) and broad-spectrum antibiotics (1x) for 8 weeks. During incubation, the culture medium analyzed by optical inverted microscope. After incubation for 8 week, to assessment of nanobacteria growth, culture medium analyzed by Scanning electron microscope (SEM).

Results: After 4 week incubation, white sedimentation at bottom of the plates observed .Analysis of optical inverted microscope showed crystal forms related to nanobacteria. SEM analysis of these White-color sediments, showed nanoparticle in size of 160 nm or less. Scanning electron micrographs showed a spherical shape of these nanobacteria. Energy Dispersive X-ray spectroscopy (EDS) also showed a pick for calcium and phosphor.

Conclusion: The growth of calcifying nanoparticles after adding the broad-spectrum antibiotics may be because of their apatite hard shells that can be supported them from the antibiotics penetration to their cells.

Keywords: Broad-spectrum antibiotics, Nanobacteria, Urinary and kidney stones

O19: **Loop mediated isothermal amplification as a rapid diagnostic method for the detection of brucella species**

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Background and Aim: A new technique called Loop-mediated isothermal amplification (LAMP) allows the rapid detection of target gene sequences without the need to expensive or complex instruments. In this study, our target region was a 196bp highly conserved fragment in the omp25 gene of Brucella spp.

Methods: This technique was performed by heating block or water bath. The specificity testing was performed using genomic DNA of the Brucella and 11 non-Brucella organisms. The products were examined by electrophoresis on 2% agarose gel and direct visual observation to appraise turbidity. For the final confirmation of the LAMP specificity, the amplified products were digested with restriction enzyme MboI.

Results: In the positive reaction, the turbidity being comprised of the white magnesium pyrophosphate precipitation was observed by naked eye after incubation at 65°C just in 60 minutes. Electrophoresis analysis showed a clear ladder-like DNA bands that is exclusively characteristic of LAMP assay, without false positive in non-Brucella organisms. Furthermore, expected produced fragments by the restriction enzyme indicated specificity of the method (70bp and 126bp).

Conclusion: The study indicated that the Brucella LAMP assay is a highly specific, inexpensive and time-saving method and can be used for the accurate detection of the organism in environmental or clinical samples.

Keywords: LAMP, Brucellosis, rapid detection

O20: **Use of Real-time PCR for Rapid Direct Detection of Mycobacterium tuberculosis Resistance to Isoniazid and Rifampicin**

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Background and Aim: The World Health Organization (WHO) estimates that approximately one-third of the world's population is infected with Mycobacterium tuberculosis. The worldwide emergence of drug resistant Mycobacterium tuberculosis has been reported in both developed and developing countries. This study evaluates a method based on real-time PCR for direct detection of the common mutations responsible for isoniazid and rifampicin resistance of Mycobacterium tuberculosis.

Methods: 83 sputum samples were collected from TB patients attending in Mycobacteriology Research Center. This study was done in 2012 to 2013. Drug susceptibility tests to Isoniazid and Rifampin were performed using Multiplex PCR method. rpoB, inhA and katG genes mutations were detected using Real-time PCR based on Taqman Assay and HRM Assay.

Results: Based on Multiplex PCR method, out of 83 samples, 47 were resistant to Rifampin and through this number, 41 cases were detected by using Taqman Assay. 36 samples were sensitive to Rifampin that 30 cases were detected by using Taqman Assay. 30 samples were resistant to both Isoniazid and Rifampin drugs. 35 samples were resistant to Isoniazid that 31 cases were detected by using Taqman Assay, and 48 samples were sensitive to Isoniazid that 41 cases were detected by using Taqman Assay. On the other hand, out of 30 samples that were studied using HRM Assay, 15 cases were resistant to Rifampin based on Multiplex PCR that all of 15 cases were detected by HRM Assay.14 samples were sensitive to Rifampin that 3 cases were detected by HRM Assay.13 samples were resistant to Isoniazid that 12 cases were detected by HRM Assay, and 16 samples were sensitive to Isoniazid that 6 cases were detected using HRM Assay. In Taqman Assay for detection of resistance to Isoniazid and Rifampin, the sensitivity 85%, 83% and the specificity 89%, 87% were derived respectively. In HRM Assay also for detection of resistance to Isoniazid and Rifampin, the sensitivity 38%, 21% and the specificity 92%, 100% were derived respectively.

Conclusion: Results of this study showed that Real-time PCR based on Taqman Assay in detection of drug resistance in Mycobacterium tuberculosis, was more sensitive than Real-time PCR based on HRM Assay. Real-time PCR besed on Taqman assay is a rapid, accurate and cost effective method in detection of Mycobacterium tuberculosis resistance.

Keywords: Mycobacterium tuberculosis, resistance, Real-time PCR

O21: **Passive Oral Immunization by Egg Yolk Immunoglobulin (IgY) to Vibrio cholera**

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Background and Aim: cholera is an intestinal infection caused by Vibrio cholera that leads to a severe diarrheal disease. The importance of hens eggs as a source of specific antibodies (IgY) is well recognized. production of Egg Yolk Immunoglobulin (IgY) against cholera organism.the main goal of this study was to production IgY antibody against cholera.

Methods: In this study, hens(25 week, n= 15) were immunized by formalin-killed Vibrio cholerae organisms using an equal volume of fround complete adjuvant. .three booster injection were given at 2-week intervals following the first injection . blood was collected from the hens and serum was isolated and immunization of hens was confirmed by indirect Enzyme Linked immunosorbent assay. On month after immunization the egg laid were collected daily for 1month and store at 4C. IgY obtained from hens immunized with formalin-killed Vibrio cholerae organisms was isolated and purified. Purification of IgY was carried out by applying 12% (w/v) poly ethylene glycol (PEG6000).

Results: SDS-PAGE analysis showed that IgY was purified. Indirect ELISA result showed high antibody titer in the serum. The purity of our purified IgY was 70% with a yield of 50mg of IgY per ml of egg yolk.

Conclusion: Because high level productions of these antibodies are easy and cost effective, IgY could be a suitable replacement of Antibiotics in treatment of Vibrio cholerae infection.

Keywords: Vibrio cholera,IgY antibody, ELISA, Egg

O22: **Presence of combined virulence genotypes of Helicobacter pylori as a risk factor for development of gastroduodenal disease in Iranian patients**

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Background and Aim: Helicobacter pylori (H. pylori), a microaerophilic spiral-shaped Gram-negative flagellated bacterium is associated with a broad spectrum of gastroduodenal diseases, such as chronic gastritis, peptic ulcers, gastric adenocarcinoma, and gastric mucosa-associated lymphoid tissue (MALT) lymphoma. Certain virulence genotypes of H. pylori have been reported to play an important role in development of severe gastroduodenal diseases. Recently, many researchers have focused on the role of synergistic effect of H. pylori virulence factors in the development of these clinical outcomes. The aim of this study was to determine any possible correlation between different subtype combinations of main virulence genes of H. pylori strains and gastroduodenal disorders in a group of infected patients.

Methods: Totally, sixty-one clinical isolates of H. pylori were obtained using cultures of antral biopsies collected from patients with different gastroduodenal diseases. PCR genotyping analysis was performed for the presence of cagL, cagA, vacA (s1/s2) and (m1/m2) alleles, iceA1/A2, babA2, and sabA genes by using specific primers. For the assessment of any possible correlation between H. pylori combined genotypes and patients' clinical data, the SPSS statistical package version 21.0 was exploited.

Results: According to the PCR results, the overall prevalence of cagL, cagA, vacA s1, vacA s2, vacA m1, vacA m2, iceA1, iceA2, babA2, and sabA genes were 96.7%, 85.2%, 75.4%, 24.6%, 29.5%, 70.5%, 42.6%, 23%, 96.7%, and 83.6%, respectively. Coexistence of multiple iceA (iceA1 + iceA2) alleles, indicating mixed infection, was found among 21 (34.4%) isolates. Overall, fifteen genotypic combinations were identified among the isolates in different gastroduodenal diseases, excluding those with mixed infections. Three genotypic combinations, cagL/cagA/vacA s1m1/iceA1/babA2/sabA, cagL/cagA/vacA s1m2/iceA1/babA2/sabA, and cagL/cagA/vacA s1m2/iceA2/babA2/sabA were determined as the most prevalent combined genotypes, mostly seen in patients suffering from severe chronic gastritis. However, no statistically significant correlation was observed between over mentioned combined genotypes and gastroduodenal diseases ($P > 0.05$).

Conclusion: H. pylori causes a life-long infection in humans with diverse clinical outcomes that may be associated with many putative bacterial virulence genotypes, host genetics, and environmental factors. Our results showed that the majority of H. pylori isolates in this study were highly virulent and carried almost all the different virulence attributes of the bacterium which are involved in different gastroduodenal disorders. Although we did not find any statistically significant relationship between genotype combinations and development of clinical outcomes. our results indicate that presence of certain virulence genotype combinations of H. pylori may at least enhance the severity of the chronic active gastritis. Moreover, some of the identified genotype combinations suggest the presence of mixed infections with divergent H. pylori strains. Finally, these genotype combination panels may also propose an important predictive tool in prognosis of disease progression in patients infected with different H. pylori genotypes, but investigation with more H. pylori strains is required to clarify this hypothesis.

Keywords: Helicobacter pylori, combined genotypes, cagL, cagA, gastroduodenal diseases

O23: **Species Spectrum of Non-tuberculous Mycobacteria Isolated from Suspected Tuberculosis Patients in different regions of Iran, identification of clinical isolates by multi-locus sequence analysis**

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Background and Aim: Identification of mycobacterium species is difficult due to a complex and rapidly changing taxonomy and the failure of 16S rRNA to discriminate many closely related species and the unreliability of phenotypic testing. We investigated a collection of nontuberculous mycobacteria (NTM) strains which isolated from suspected tuberculosis patients at Tuberculosis Reference Centre (Ahvaz, Iran) and Masoud Laboratory (Tehran, Iran) during 2008-2012 to evaluate the species spectrum of NTM isolates.

Methods: The isolates were homogeneous by preliminary biochemical and phenotypic characterization, however were heterogonous by hsp65-PRA method. A representative isolate from each hsp65-PRA pattern, were subjected to identification using single locus and multi locus sequence analysis (MLSA) based on 16S rRNA , rpoB, hsp65 and 16S-23S internal transcribes spacer (ITS) fragments to determine their taxonomic affiliations.

Results: All NTM isolates from different clinical specimens considered as etiological agents causing disease according to American Thoracic Society (ATS) guideline. Phenotypic evaluation alone assigned 66 (72%) isolates to a species or complex and 81 (83%) isolates showed previously reported hsp65-PRA patterns. Although sequence base identification using single locus such 16S rRNA, rpoB, hsp65 or ITS identified the isolates to species level, MLSA correctly identified 16 different species of NTM from clinical isolates.

Conclusion: In summary, four-locus MLSA is a reliable method of elucidating taxonomic data and reliable species identification of mycobacterium isolates and would be more feasible for routine use in Tuberculosis (TB) reference laboratory.

Keywords: Mycobacterium, infection, identification, multi locus sequence analysis (MLSA)

O24: **microbiological evaluation of Poultry paste**

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Background and Aim: A wide variety of Food products have poultry paste in their ingredients. Poultry paste usually is contaminated with some pathogen bacteria during not suitable preparation. Due to this fact and more probability to microbial contamination, this study was accomplished to control microbial quality of poultry paste using as a major component in many kinds of foods that is also an important contaminant agent.

Methods: this study was fulfilled based on the national standard (NO. 2946-1810-6806-6805) for detection of E.coli, E.coli O157: H7 , Salmonella and Staphylococcus aureus in 60 samples .

Results: Findings of the survey showed 58 (96/66%) of the total samples were contaminated with at least one of studied bacteria and weren't appropriate to use , 53(88%) have E.coli, 5 (8%) have Salmonella, , 14 (23/33%) have S.aureus and 2(3.3%) have E.coli O157: H7.

Conclusion: Results indicated that the method of production and distribution of this primary product don't have good quality , and since various reports claim of inappropriate health quality of final products , more supervisions and more serious of health authorities are required to poultry paste production to improve the safety of respect foods.

Keywords: microbiological quality , poultry paste , contamination

O25: **Use of One-Plate Test (A microbial growth inhibition assay) as a screening test for the detection of antimicrobials residues in chicken's meat**

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Background and Aim: The intensive production of chicken's meat has led to an extensive use of antimicrobials in poultry farms for growth enhancement and feed efficiency in addition to treatment and prophylaxis of infectious diseases. The use of antimicrobial drugs in animals has recently become an important public health issue. The increased use of such compounds has showed many harmful effects on consumer such as, stimulation of microbial resistance and thus increases the risk of foodborne infections when such antibiotic resistant pathogenic bacteria enters the food chain. Detection methods based on microbial inhibition are able to detect a wide range of antimicrobials although they lack specificity. A one-plate screening method is described for the microbiological detection of antibiotic residues by growth inhibition of Bacillus subtilis in agar medium, at pH 7. The aims of present study were to: (i) introduce of One-Plate Test as a rapid and sensitive screening test for identification of antimicrobial residues in chicken's meat, (ii) determination the distribution of antimicrobial residues in various edible poultry tissues which locally produced and marketed in Kermanshah province (iii) investigate the effect of season on persistence of these antimicrobial residues in chicken's meat.

Methods: Eighty-eight poultry carcasses (88) were collected from five different poultry meat providing centers of Kermanshah province in west of Iran during winter and summer 2012. The culture medium of Muller Hinton Agar (MHA) was prepared and autoclaved. The pH was adjusted to 7±0.05. When the medium had cooled to 50°C, a spore suspension containing 104 Bacillus subtilis (ATCC 6633) spores per ml were added. The chicken frozen samples including the breast, liver and tight were homogenized and then centrifuged at 10,000g for 15 min. Ten microliters of supernatant for each sample was applied to paper discs which were put on the earlier prepared and seeded agar plates. After incubation at overnight at 30 °C, an inhibition zone of 2 mm with one of the paper disks was considered as a positive result. SPSS software (chi-square) was employed to statistically evaluate the data.

Results: The OPT test showed that out of 264 samples, 50 (18.9%) were positive as demonstrated the diameter of inhibition zone higher or equal than 2 mm. The maximum percentage of positive sample observed in livers (39 samples, 44.3%), tight (7 samples, 8.0%) and breast muscles (4 samples, 4.5%) respectively. The results show that there are significant differences between various tissues ($P < 0.05$). As shown in this study, the high concentration of antimicrobial substances can be accumulated in the liver raising awareness for more cautious consumption of this organ. Moreover, Levels of antimicrobial residue in samples collected in winter were significantly higher ($P < 0.05$) than those collected in summer.

Conclusion: The OPS has many advantages because it provides a lot of information and is simpler and cheaper to perform from other screening tests.

Keywords: One-Plate test, antimicrobial residues, Chicken, Kermanshah.

O26: **Evaluation Of Crude Sesquiterpenoid Extract Of Ganoderma reissi as A Natural Food**

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Background and Aim: Ganoderma is a popular remedial mushroom, which has been used in the traditional medicine for the prevention or treatment of a variety of diseases. In the present study we evaluated the inhibitory effect of sesquiterpenoid extract of Ganoderma reissi on fungal and bacterial growth in different media.

Methods: Microbial growth inhibition by sesquiterpenoid extract of Ganoderma reissi was evaluated in the media with natural ingredient such as milk agar (MA), tomato juice agar (TJA), wheat flour agar (WFA) and pine apple juice agar (PAJA) with or without adding Ganoderma sesquiterpenoid extract, sodium benzoate alone or Ganoderma sesquiterpenoid extract along with sodium benzoate. The pH of the media was adjusted to 6. Three strains of bacteria viz. Bacillus subtilis, Eschericea coli, Staphylococcus aureusand one strain of fungus i.e. spergillus niger were used. The bacterial strains were maintained on nutrient agar medium while A. niger on Sabouro-dextrose-agar.

Results: The sesquiterpenoid extract of Ganoderma at 0.2% concentration significantly inhibited the growth of all microorganism on natural media ($P<0.01$). These results were comparable with inhibitory effect of sodium benzoate at 0.1%, and Ganoderma extract 0.1% plus sodium benzoate 0.05%. The sodium benzoate at 0.2 % concentration completely inhibited the growth of all microorganisms. Similarly combination of Ganoderma extract (0.1%) and sodium benzoate (0.05%)] fully inhibited the growth of all microorganisms in all media ($P<0.01$).

Conclusion: Considering the findings of this study, and considering the side effects of synthetic preservative, crude sesquiterpenoid extract of Ganoderma reissi might be used as an appropriate food preservative.

Keywords: Food preservative, Ganoderma reissi, Sesquiterpenoid

O27: **Biofuel production in cyanobacteria by system biology methods**

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Background and Aim: There has been an enormous increase in the global demand for energy in recent years as a result of industrial development and population growth. Biofuel is one of the best resources because is cheap, abundant, renewable and clean. By modification in metabolic network of cyanobacteria we can product biofuel. There are two general strategies for modification of cyanobacteria: Biotechnological methods and system biology methods. The purpose of this study was to design new metabolic network in cyanobacteria by system biology methods.

Methods: In the first step, according to articles and databases, a new metabolic network was reconstructed. The theoretically, this metabolic network could product biofuels such as ethanol, isobutanol and etc. In the next step, the constructed metabolic network converts to SBML and import in COBRA toolbox in MATLAB. In final step, the flux balance analysis (FBA) was run.

Results: Quantitative results are shown that modified cyanobacteria can grow and product biofuel. Because FBA outnumber of zero. Organisms, and all other metabolic systems, require some input of nutrients. When FBA equal zero means that nutrients that are not present or not absorbed by the organism do not enter its metabolism.

Conclusion: It is expected that the system framework developed in this study would be useful for the in silico design of novel metabolic pathways to be employed for the efficient production of chemicals, fuels and materials. So we can modify cyanobacteria by Biotechnological methods according to new metabolic pathways that designed by system biology methods.

Keywords: cyanobacteria; biofuel; system biology; metabolic network; F

O28: **Production of pentavalent Clostridial toxoid vaccine and its comparison to conventional bacterin vaccine**

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Background and Aim: Genus Clostridium, gram positive spore forming anaerobes are very important bacteria for human and animals pathogenesis. Clostridial diseases frequently occurs In Iranian farms and different Clostridium species are responsible for high mortality in domestic animals like as sheep and goats.

Methods: in the present study, three groups of experimental vaccines were produced and comprised to each others. 1st group as bacterin pentavalent vaccine which produced in 20 liters glass vessels and adsorbed on aluminium hydroxide adjuvant. 2nd group as bacterin pentavalent vaccine produced which in 12 liters fermenter and adsorbed on aluminium hydroxide adjuvant. 3rd group as bacterin-toxoid pentavalent vaccine which produced in 12 liters fermenter and adsorbed on aluminium hydroxide adjuvant. Freedom from abnormal toxicity, Safety and potency tests were done for all groups of vaccines.

Results: Results showed that the 3rd group vaccine which was produced by fermenter and purified by ultrafiltration system is the best choice for scale up.

Conclusion: now we are getting prepared for mass production of this vaccine in Iran

Keywords: pentavalent vaccine, Clostridium, toxoid, fermenter, ultrafiltartion

O29: **Application of hyperthermophilic Geobacillus sp. Isolated from Larijan hot spring for removal of paraffinic oil-well deposits**

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Background and Aim: Formation of deposits in oil reservoirs is a very common phenomenon that reduces oil production or even stops the flow of oil. The aim of this study was to isolate hydrocarbon-degrading bacteria, effective in removal of oil deposits.

Methods: Samples were gathered from several naturally enriched oil contaminated hot environments. Mineral base media containing solid paraffin as sole source of carbon was used for isolation of thermophilic bacteria at 70˚C. The isolated strains were screened for long chain alkane degradation, and acid and biosurfactant production.

Results: Among 31 isolated thermophilic strains, four were selected for further investigation based on their potential for paraffin degradation, and biosurfactant and acid production. Homology analysis of 16S rDNA sequences revealed that all four strains belonged to the genus Geobacillus. Two of the four selected strains were able to degrade paraffin, icosane and tetracosane and the other two strains could produce acids and biosurfactants during growth on molasses. To investigate the ability of the selected strains in oil deposits removal, a model cylinder was made from a 5-inch oil well tubing. The wall of the model was covered with two different oil deposits (with paraffinic and asphalthenic nature). Pure bacterial cultures in fresh media were added to the model and were incubated in microaerophilic conditions at 70˚C for two weeks.

Conclusion: The results showed that the strains were able to remove more than %70 of paraffinic deposit; however asphaltenic deposits were persistent against microbial treatment. Analysis of the microbial released oil deposit compositions revealed that the amount of aromatic fraction decreased due to microbial degradation, while the amount of saturated fraction increased.

Keywords: Geobacillus; Long chain Alkanes; Wax deposition; Biodegradation; Hyperthermophile

O30: **Molecular characterization of biodegradable Mycobacteria from hospital water in Iran**

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Background and Aim: Background: Biodegradation is a treatment process that naturally occurs by some microorganisms as well as plants to break down hazardous substances that pose environmental and in particular human risks. Due to safety and convenience of this process, it has become an accepted approach for cleaning polluted environment. Some microorganisms, mainly members of two genera i.e., Pseudomonas and Mycobacterium, were found to be capable of transforming and degrading polluting agents. The aim of present study was to isolate and characterize mycobacteria from hospital water with ability to biodegrade organic and inorganic compounds.

Methods: Materials and methods: The samples were collected from hospital water supplies. Isolation of mycobacteria was carried out according to standard methods. The colonies were subcultured on Lowenstein Jensen (LJ) medium to obtain a pure culture. Ziehl-Neelsen (ZN) staining was used to confirm presence of acid fast bacilli. The Mycobacterium-specific PCR amplification protocol targeting a 228-bp region of the 65-kDa heat shock protein (hsp) gene confirmed belonging of the isolates to the genus Mycobacterium. Further amplification and direct sequence analysis of almost full length of 16S rDNA gene confirmed the true identity of the isolates at species level.

Results: Results: The isolates, AW18-1, AW18-2, AW18-3, AW27-2 and AW27-6 were recovered after direct culture of water samples and fully characterized based on phenotypic and molecular characteristics. The almost complete 16S rDNA gene sequences of the isolates AW18-1 and AW18-3 were identical and showed 99.4% similarities with M. fredriksbergense. The isolates AW18-2, AW26-2 and AW27-6 showed 99.5%, 99.7% and 99% similarities with M. austroafricanum, M. obuense and M. phocaicum.

Conclusion: Conclusion The Iranian isolates were found to be M. fredriksbergense, M. austroafricanum, M. obuense and M. phocaicum. These species have been identified as mycobacteria with capability of biodegradation. M. fredriksbergense is the important species of polycyclic aromatic hydrocarbon (PAH)-degrading (pyren). M. austroafricanum can degrade 2-ethylhexyl nitrate (2-EHN, a major additive of fuel which is used to comply with the 21 cetane number of diesel and isooctane. M. phocaicum can degrade Fluoroglycofen ethyl (a kind of diphenylether herbicide) and M. obuense is a petroleum biodegradation organism. The isolation of these biodegradation mycobacteria from hospital water might indicate the role of harsh ecology in natural selection of organisms with specific survival capability. It is clear that chemotaxis is a selective advantage to the degradative bacteria for guiding them to sense and locate pollutants that are present in the environment. Our findings showed that although the presence of such mighty microbes in hospital water could be considered as a hazardous source of infection for patients and in particular those with debilitating underlying conditions, however the hospital niche could be a potential ecology for screening and spotting very useful organisms with capability of degrading polluting agents.

Keywords: Biodegradation, Mycobacterium, 16SrDNA, water supply

O31: **Analyzing indigenous hydrocarbon-utilizing bacteria of an oil-contaminated soil to assess the potential for field bioremediation process**

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Background and Aim: Bioremediation process is advantageous owing to the cost-effectiveness and environmental friendly nature of the technology and has been greatly used in hydrocarbon mitigation. According to the ubiquity of hydrocarbon-degrading microorganisms, this study was devised to identify indigenous hydrocarbon-utilizing bacteria from an oil-contaminated site in Siri Island, Persian Gulf.

Methods: Soil samples were collected and enriched in Bushnell-Haas mineral medium amended with 2 g/l hexadecane. After 30 days of incubation at 30° C, enriched cultures were streaked on R2A agar plates and distinct colonies were purified by successive cultivations.For phylogenic identification, DNA was extracted from each bacterial culture and 16S rRNA gene was amplified.

Results: Fifty pure bacterial strains were obtained, among which only fourteen strains were able to grow in hexadecane-containing minimal medium. Obtained 16S rRNA gene sequences were submitted in GenBank (accession numbers JX500266 to JX500279). According to 16S rRNA sequence analysis, it was shown that the isolated strains belonged to the genera Gordonia, Microbacterium, Phenylobacterium, Nocardioides, Bordetella, Pseudomonas, Staphylococcus and Rhodococcus. Interestingly one of the isolated strains had only 93.95 % of sequences similarity to the type strains of Rhodoplanes pisinae and could possibly be introduced as a new genus. Considering the high diversity of hydrocarbon-degrading bacteria, to evaluate the potential of soil biostimulation, total culturable heterotrophic bacteria and hydrocarbon-utilizing bacteria of the soil were enumerated by standard plate count and MPN methods, respectively. After stimulation of bacterial growth through establishing improved nutrient and aeration condition, total culturable heterotrophic bacterial count reached from 3.5 × 106 to 9.2 × 108 CFU/gr and the population of hydrocarbon-utilizing bacteria increase from 2.9 × 104 to 9.3 × 107 CFU/gr.

Conclusion: These results showed that the initial count of hydrocarbon-utilizing bacteria was not sufficient for a promising bioremediation process; however their growth was specifically promoted 3200 times after stimulation, in a way that their relative frequency increased from 0.8 % to about 10 % of the total heterotrophic bacteria. Consequently, it was shown that a diverse hydrocarbon-degrading microbial flora resided in the investigated soil which could be exploited in bioremediation process.

Keywords: hydrocarbon-utilizing bacteria, oil-contaminated soil, 16S rRNA, phylogenetic,bioremediatio

O32: **Detailed identification of desert-originated bacteria carried by Arabian dust storms to Iran**

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Background and Aim: The species diversity of desert-originated bacteria carried by Arabian dust storms to Ahvaz, Iran was examined by using conventional phenotypic and molecular tests during dusty and clear days in 2011–2012 at four sites in Ahvaz urban and suburban area.

Methods: A total of 1500 strains of bacteria which was isolated from dusty and clear days were subjected to identification by phenotypic methods and subsequently clustering by PCR restriction fragment length polymorphism analysis (PRA) and speciation by 16S rRNA gene sequence analysis.

Results: The isolates were heterogeneous and mostly were unidentifiable by biochemical tests. A representative isolate from 74 different cluster of 16S rRNA-PRA pattern was identified by 16S rRNA gene sequences analysis. Sequence analysis revealed 74 different species mostly belongs to species of genus Bacillus and Actinomycets. Some species of desert-originated bacteria that identified have been reported as human and plant pathogen mainly in immunosuppressive hosts.

Conclusion: Diversity of bacteria species suggests that dust events in the Middle East might be have a significant effect on the airborne microbial populations, which might impact on health, agriculture, and ecology.

Keywords: identification, desert-originated bacteria, dust storms, Iran

O33: **Monitoring the copy number of two functional genes, AlkB and C23DO during biodegradation of PAHs and Alkanes**

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Background and Aim: Siri is an oiliproducing island in the Persian Gulf, Iran. An area of 4 hectares in the island has been contaminated by oil sludge during 1980s. So there is an increasing need for cost-effective remediation technologies such as bioremediation for hydrocarbon contamination. Since crude oil is mostly composed of hydrocarbons especially alkanes and PAHs (poly aromatic hydrocarbons), the copy number of AlkB(Alkane monooxygenase)and C23DO(Catechol 2,3 dioxygenase) genes (as indicator of active biodegradation of alkanes and PAHs in soil) was studied using Real-Time PCR technique during bioremediation of alkanes and PAHS.

Methods: Four microcosms were prepared using Siri soil including: control (without any contaminant), Alkane (composed of 1%W/W C13 to C20 alkanes), PAH(composed of anthracene, phenanthrene, fluoranthene, pyrene (each 100 ppm) and benzo[a]pyrene (20 ppm)) and a combination of PAHs and alkanes. Optimum conditions for bioremediation such as humidity and sufficient nitrate and phosphate were afforded. The microcosms were kept in room temperature during a six-month survey period. Sampling was done twice a month for both chemical and biological analysis. Chemical analysis included GC (for detection of the amounts of residual alkanes) and HPLC (for detection of the amounts of residual PAHs). Biological analysis included heterotrophic bacterial count and relative quantification of AlkB and C23DO genes according to Pfaffl et al. method.

Results: Results of heterotrophic bacterial count indicated a logarithmic increase of bacterial count during the first week. This increase was coincident with the decrease of both alkanes and PAHs. Results of Real-Time PCR showed that after six month the copy number of AlkB gene increased two times in comparison to the start time in alkane microcosm, and 17 times in PAH alkane microcosm. Also the copy number of C23DO gene increased 3 times in PAH microcosm and 17 times in PAH+alkane microcosm.

Conclusion: The increase of copy number of the key functional genes; AlkB and C23DO in soil microcosm is an indicator of enrichment of hydrocarbon degrading bacteria. Also there is a significant relationship between the copy number of these genes and elimination of the contaminants as indicated by GC and HPLC analyses. The results indicate that the copy number of both genes more significantly increased in the microcosm that contains a combination of PAHs and alkanes, than the other microcosms. From these findings we may conclude that the alkanes and PAHs are co-metabolically degraded in soil. Additional study on identification of microorganisms involving in petroleum hydrocarbon biodegradation, can improve our insight of the "role of indigenous bacteria of soil in bioremediation".

Keywords: bioremediation- petroleum hydrocarbons - AlkB gene - C23DO gene - Real-time PCR

O34: **Cadmium and Nickel Bioremediation by radiation resistant bacterium**

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Background and Aim: All ionizing radiations, at sufficiently large exposures, can cause cancer and if the radiation waste water contains heavy metals, the risk of this type of waste has multiplied. Removal of heavy metal ions from environment is essential due to their high peril such as high level of toxicity, persistent in the environment and their carcinogenic and mutagenic properties .Heavy metal ions from wastewater are commonly removed by conventional physico-chemical methods, such as chemical precipitation , ion exchange , evaporation and sorption but these methods have several disadvantages , such as generally very expensive , incomplete metal removal and biological incompatibility; therefor ,need for innovative treatment technologies for removal heavy metal is necessary .In recent years, research attention has been focused on biological methods due to their advantage such as the ability of in-situ application in situ at the contaminated site, cost effective and eco-friendly. Microorganisms such as alga, fungia, bacteria are potent bioremediator for removal heavy metal via various uptake mechanisms. Microorganisms such as alga, fungia, and bacteria are potent bioremediator for removal of heavy metal via various uptake mechanisms.

Methods: Bacterial strain isolated from hot springs springs were screened on TGY broth medium and were incubated at 30o C and 180 rpm shaking for 3 days in Incubator by 180 rpm shaking and then spread 100 microlitter of dilution series of sample on trypton glucose yeast extract TGY plate . Plate was put under UV light with an intensity of 15 joules. The grown colonies on the plate are radiation resistant strain. Minimum inhibitory concentration (MIC) of heavy metals (Cd and Ni) were determined at 30oC for 24 hours by microtiter plate . Heavy metal bioremediation were analyzed by Atomic Absorption instrument and bacterial 16S rDNA was amplified by using the universal bacterial 16S rDNA primers.

Results: Sequencing indicated the bacterium is Kocuria rosea .In normal condition OD growth for Kocuria is 1.048 (at A 660) but in present of Cd and Ni Minimum inhibitory concentration OD is 0.446 (at A 660) for Cd and 1.070 (at A 660) for Ni. This strain show it can remove 60 Percent and only 5 Percent of Ni and Cd respectively.

Conclusion: As a result, it can be concluded that K. rosea can be used in removal of Nickel ion from radioactive wastewaters.

Keywords: Radiation resistant strain, Heavy metal, Wastewaters, Bioremediation

O35: **The Population Structure of Persian Salmonella enterica Enteritidis analyzed by Multi Locus Variable number tandem repeat Analysis**

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Background and Aim: Salmonella enterica subsp. enterica serovar Enteritidis is a leading cause (55%) of salmonellosis worldwide. Chicken and environment are natural habitat of S. Enteritidis with a globally-recognized importance as a zoonotic pathogen. Poultry, especially those related to food products (poultry meat & raw eggs) are principle reservoir of S. Enteritidis. In Iran despite holding one of the largest chicken industries in the region, epidemiology of S. Enteritidis at molecular scale is just poorly understood. S. Enteritidis is the most clonal serotype, so powerful typing strategies for surveillance and studying outbreaks are required. MLVA is a powerful genotyping method based on the amplification of DNA fragments that contain Variable Numbers of Tandem Repeats (VNTR). To explore genetic structure of S. enterica Enteritidis population in Iran, we evaluated 3 previously-reported VNTR markers including SE5, SENTR4 and SENTR7.

Methods: By literature review, most commonly used loci which recommended as a standard system for genotyping of S. Enteritidis were identified. This MLVA genotyping system was applied on 80 S. Enteritidis isolates representing a diverse geographical (Fars, Qazvin, Markazi, Zanjan, Khorasan Razavi) and host (chicken and human) origin. Genomes of the isolates were successfully PCR amplified at three loci. To improve precision of gel electrophoresis findings, some of PCR products were sequenced.

Results: To our surprise, limited genetic diversity was observed in the study panel where only 5 MLVA types were detected. SE5 with 5 alleles produced the highest discriminating capability followed by SENTR7 and SENTR4 where 2 alleles were displayed by each locus. To compare the diversity between MLVA loci, Simpson's diversity index of the three-loci (SE5, SENTR4, and SENTR7) was obtained according to Hunter and Gaston. Polymorphism rates were 0.41, 0.09 and 0.05 for SE5, SENTR7 and SENTR4 loci markers, respectively.

Conclusion: While we agree with other researchers on usefulness of MLVA as a highly discriminative genotyping system, we assume discerning clones and subclones of S. Enteritidis populations cannot be necessarily provided by the same set of MLVA loci. To clarify, while SE5 perfectly works to classify S. Enteritidis isolates in the Iranian environment it has a low credit in the standardized MLVA typing scheme recently developed in Europe. This might be due to differentiation between the population genomic structure of the Iranian Enteritidis isolates and European population which studied before. A strong association between human and chicken cases was detected, because three of five MLVA types found in this study, shared by both of them.

Keywords: Salmonella Enteritidis, MLVA, Genotyping, Clone, Discrimination, Epidemiology, SENTR4, SENTR7, SE5

O36: **Sequencing & phylogenetic analysis of the vlhA gene of Mycoplasma synoviae isolates from commercial poultry flocks**

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Background and Aim: Mycoplasma Synoviae (M.Synoviae) is a major avian pathogen and cases many economic losses on poultry industry in Iran, Therefore, early and reliable diagnosis and also strain differentiation is a key to prevention and control. The aim of our study was the variable lipoprotein and haemagglutinin (vlhA) gene sequencing and phylogenetic analysis of Iranian M.Synoviae isolates from commercial poultry flocks and comparison of the vlhA gene sequences of Iranian isolates with vlhA M.Synoviae sequences of other contries.

Methods: Samples were collected from 3 central province of commercial broiler chicken farms (Tehran, Markazi and Qazvin). The samples were obtained from flocks with clinical signs of infection by M.synovie. In study tow published primer sets were used for the specific detection of genus and species of M.synovie.

Results: Of the 43 swabs 33 yielded one of the potentially pathogenic Mycoplasmas evaluated for Mycoplasma genus using PCR method and 24 of the swabs yielded M.synoviae using vlhA-PCR method. Using oligonucleotide primers complementary to the single-copy conserved 5´ end of vlhA gene, amplicons of ~400 bp (base pair) were generated from 24 isolates of M.synoviae. In the present study, the conserved domain of the vlhA gene M.synoviae was sequenced and analysed for 10 isolates of M.synoviae isolated from Iran and also compared with vlhA M.synoviae sequences than have been available in GeneBank. These data indicated that, there was a complete concordance between all Iranian isolates nucleotide sequence. DNA sequence analysis was used to determine the phylogenetic relationships among the M.synoviae isolates and the tree showed which all 10 isolates of M.synoviae isolated from Iran were most closely related to sequences of M.synoviae of Japan, as well as, the sequence obtained from all Iranian isolates with the sequences of the isolates from Japan were classified into the same phylogenetic cluster. This study demonstrated differentiate between Iranian isolates and vaccine strain and be observed in all Iranian M.synoviae isolates, point mutations (transition and transvertion) and frame-shift mutation (deletion and addition) in comparison with vaccine strain, also vaccine strain clustered with none of the Iranian isolates that were found in study.

Conclusion: PCR for M.synoviae using primers located in the vlhA gene is a sensitive and specific test for detection of M.synoviae and an efficient tool for primary strain differentiation and its value for strain typing.

Keywords: M.synoviae; vlhA; phylogenetic; mutation; vaccine strain.

O37: **molecular and clinical study on prevalence of Feline Herpesvirus1 and Calicivirus in correlation with Feline Retroviral infections in healthy and in cats with respiratory disease symptoms in Tehran**

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Background and Aim: Upper respiratory tract disease (URTD) ('cat flu') is still a common clinical problem in cats worldwide. The main primary pathogens include feline herpesvirus type 1 (FHV) and feline calicivirus(FCV). Also, Feline immunodeficiency virus (FIV) and Feline leukemia virus (FeLV) are among the most common infectious diseases of cats which suppress the immunity system of cats and make cats susceptible to URTD. The aim of this study was to determine the prevalence of FHV-1 and FCV in non vaccinated clinically healthy cats and those with URTD. To know the prevalence rate of retroviruses in cat population and also understand the immunosuppressive effect of retroviruses on disease progress in sick cats we investigated the prevalence of FIV and FeLV in both populations.

Methods: Oropharyngeal and conjunctival swabs and blood samples were taken from 16 cats with clinical signs of URTD and 26 clinically healthy cats. Reverse transcription polymerase chain reaction (RT-PCR) performed to diagnose FCV and FeLV and polymerase chain reaction (PCR) to detect FHV-1 and FIV infections.

Results: FCV was detected in all cats with URTD and 87% of them were positive For FIV and 93% for FeLV. FHV rate of infection was 43% in sick cats. In clinically normal cats Prevalence rates of FCV and FHV were about 50%, but FIV and FeLV rates (42% and 65% respectively) were high in comparison to other reports. Stomatitis was observed in 50% of cats with URTD. In 50% of cats with corneal ulcers, FCV was detected alone, while we expected it in correlation just with FHV-1 infection.

Conclusion: It seems that cats are getting infected with new variants of Calicivirus which have more virulence and are able to damage other uncommon tissues like cornea and conjunctiva or maybe these various signs are due to effect of retroviral infections. The high prevalence of FIV and FeLV may be due to existence of large population of stray cats in Tehran.This is the first molecular study of FeLV and FCV in Iran and finally, it seems that rate of FCV and FHV-1 prevalence in FIV or FeLV infected cats is more than other non infected ones.

Keywords: FCV, FHV-1, prevalece, corneal ulcers, feline leukemia virus,feline immunodeficiency virus, respiratory disease

O38: **Efforts to SNP typing of Persian Bacillus anthracis, from dream to reality**

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Background and Aim: Annually, over 50 million doses of Anthrax vaccine are used in Iran to protect the national cattle, sheep and goat herds against anthrax. This is a huge national surveillance scheme however, large and small outbreaks of the disease occur every year where occasionally human cases are affected. Why is that? Are these epidemiological events reflecting vaccination failures or there are actually new strains that escape the immunity protection provided by this vaccine? This will not be answered unless population structure of B. anthracis in Iran is well studied in the first place. Single Nucleotide Polymorphism (SNP) analysis focusing on 13 evolutionary meaningful loci is currently the most globally-used genotyping strategy to understand history and today's population structure of B. anthracis. The work presented here is a description of the most recent findings of SNP typing project on the Iranian B. anthracis population.

Methods: An in situ investigation was conducted to locate the 13 previously-reported SNPs within the whole genome of Bacillus anthracis Sterne 34F2. A 500 bp-long portion of the bacterium genome on each side (forward and reverse) of all SNPs were selected and used to design primers suitable for traditional PCR amplification. PCRs including ingredients and amplification protocols were set up and adjusted in a way that 13 individual SNP reactions could be performed in a single PCR run. PCR products from al the isolates were sequenced and the target nucleotide at each SNP locus was identified. This strategy was conducted on 8 Iranian isolates

Results: The original SNP typing version adopted by American researchers is expensive and so far impractical as it requires advanced laboratory equipments and expertise. Simplification of the whole typing system devised here was fully successful. High quality PCR products were produced from all the tested isolates and sequenced. Combinational SNP patterns of the 8 Iranian isolates were subsequently achieved.

Conclusion: The technical "know-how" of Anthrax SNP typing is now established at Razi Vaccine & Serum Research Institute. We know what major SNP groups of B. anthracis are circulating in Iran. This work is still ongoing to cover new isolates from more geographical areas of the country.

Keywords: Bacillus anthracis,SNP typing,Persian

O39: **Molecular characterization of wild life and laboratory Burkholderia mallei isolates from Iran**

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Background and Aim: Glanders caused by Burkholderia mallei, is an zoonotic disease. Globally, mallein test is the most frequently-used method in diagnosis of glanders., mallein is still prepared of standard B.mallei strain(s) In order to characterize the genomic properties, we have studied nucleotide structure of 23SrDNA, flip and BimA genes of an B.mallei strain that is used for preparation of mallein at the Razi Vaccine and Serum research Institute and also a B.mallei isolate collected from a diseased tiger linked to the latest glanders outbreak at Tehran zoo.

Methods: Cultures of all bacteria were grown in Tryptic Soy bean Broth and Tryptic Soy bean agar for 72 hours at 37°C. PBS-prepared bacterial suspensions were used to subcutaneously inoculate two guinea pigs in order to assess capability of bacteria to develop Strauss phenomenon.Genomic DNA was extracted by using Isoamyl alcohol-Chloroform method. Genomic work included 4 PCRs conducted using 3 primer pairs specific to B.mallei and a single primer pair shared by both B mallei and Burkholderia pseudomallei. PCR products were sequenced and these were matched against their corresponding genomic loci from the fully-genome sequenced strain of B.mallei to enable comparative analysis of findings

Results: Both inoculated guinea pigs developed the characteristic Strauss phenomenon The productive strain and tiger isolate demonstrated observably different phonotypical properties in their virulence and culture. PCR findings re-confirmed the identity of both productive strain and tiger isolate as B. mallei while their genomic structure at the examined loci was found to be fully identical with one another and also with the B.mallei strains in gene bank

Conclusion: This strategy represented a diagnostic algorithm suitable for differentiation between B. mallei and B. pseudomallei Because importance of disease and limitation of mullein test in clinical and advanced cases of glanders using of sensitive and rapid diagnostic methods dependent on PCR and aknowledge of epidemiology molecular of glanders, too is useful

Keywords: Burkholderia mallei, Glanders, Mallein, flip gene, BimA gene, 23SrDNA gene

O40: **Helicobacter pylori genes expression close to in vivo conditions**

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Background and Aim: In studies of H.pylori genomics, in the first, Gastric biopsy specimens culture then bacterial genome extracted commonly. Before and after culture, H.pylori gene expression Levels is different. It is reasonable survey of H.pylori gene expression must be in vivo or close to in vivo conditions. The aim of this study was to survey of H.pylori gene expression without culture and close to in vivo conditions. In other words, however in the most cases, biopsy specimens have trace amounts of H.pylori genome, survey of H.pylori gene expression performs on biopsy specimens directly and without culture.

Methods: Urease-positive biopsy specimens were placed in RNAlater solution immediately. Extraction of RNA Total from biopsy tissue was performed with using the Tripure Kit rapidly. Produced Total RNA Measured with using Nanodrop and round on 1% agarose gel. The cDNA was synthesized from RNA molecules with using the Kit. cDNA product was trace so to fix this problem we designed an initial PCR with using Real-Time PCR primers (tip-alpha gene primers and ureA gene primers). The initial PCR was done with using same amount of initial cDNA in all of reactions. Real time RT PCR was done with using initial PCR products. The Standard curve and the ΔΔCT methods were used to measure PCR Efficiency and detection of genes expression levels accordingly. In ΔΔCT method, it is assume the PCR efficiency to be the same for all of reactions.

Results: Non-specific amplification was not created in Initial PCR products and amounts of cDNA after Initial PCR were increased sensitivity of Real Time RT PCR.

Conclusion: An initial PCR program on trace amounts of initial cDNA that synthesized from biopsy specimens total RNA, can increasing real time RT PCR sensitivity in survey of bacterial gene expression without culture.

Keywords: Helicobacter pylori, Real Time RT PCR, In Vivo

O41: **Detection of Wolbachia pipientis, including a new strain containing the wsp gene, in two sister species of Paraphlebotomus sandflies, potential vectors of zoonotic cutaneous leishmaniasis**

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Background and Aim: Individual, naturally occurring Phlebotomus mongolensis and Phlebotomus caucasicus from Iran were screened for infections with the maternally inherited intracellular Rickettsia-like bacterium Wolbachia pipientis via target¬ing a major surface protein gene (wsp). The main objective of this study was to determine if W. pipientis could be detected in these species.

Methods: The sandflies were captured from different regions of Turkmen Sahara, Iran, genus and spices were identified using standard keys. Phlebotomus mongolensis and Phlebotomus caucasicus were screened using polymerase chain reaction to amplify a fragment of the Wolbachia surface protein gene. The obtained sequences were edited and aligned with database sequences to identi-fy W. pipientis haplotypes.

Results: Two strains of Wolbachia were found. Strain Turk 54 (accession EU780683) is widespread and has previously been reported in Phlebotomus papatasi and other insects. Strain Turk 07 (accession KC576916) is a novel strain, found for first time in the two sister species. A-group strains of W. pipientis occur throughout much of the habitat of these sandflies. It is possible that Wolbachia is transferred via horizontal transmission.

Conclusion: Horizontal transfer could shed light on sandfly control because Wolbachia is believed to drive a deleterious gene into sandflies that reduces their natural population density. With regard to our findings in this study, we can conclude that one spe¬cies of sandfly can be infected with different Wolbachia strains and that different species of sandflies can be infected with a common strain.

Keywords: Wolbachia pipientis - wsp gene - Paraphlebotomus - L. major - Iran

O42: **Potential of plant-derived antimicrobial agents**

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Background and Aim: Seeking natural anti-infectious agents in plants is an ancient idea. Worldwide spending on finding new antimicrobial compounds is expected to increase 60% over the next two decades (Cowan, 1999). Although there is an estimated 8000 plant species in Iran (UNEP, 2004), less than 2% have been investigated for their biological activity. As such, there exists a huge potential for discovering novel germicidal candidates amongst plant-derived compounds. A recent analysis of inhibitory (anti-bacterial, fungal, parasitic and viral) drugs over the period 1981–2002 revealed that close to 70% are naturally derived (Gurib-Fakim, 2006). A review on the number of articles published on the antimicrobial activity of plants during two periods 1966-1994 and 1995-2004 showed a near threefold increase from 115 to 307 (Rios and Recio, 1995). A large number of bioassay is available for the testing of plant extracts and isolated compounds. Since these assays are relatively expensive, it is essential to have the know-how to set them up, perform them, interpret the data sets generated from them and be aware of the limitations of the chosen methodology (Cole, 1994).

Methods: A survey of published data on the antimicrobial activity of plant extracts demonstrates an increased interest for this type of work among researchers. Antifungal and antibacterial bioassays of plant-derived compounds (phytochemicals) are reviewed in this paper. Data interpretation from such assays are examined. The value of these assays in terms of methodology is discussed.

Results: Although this type of research is most common, the methodology is often weak. In methodology, only vouchered microorganisms should be used and the strain should always be quoted in the documentation following isolation. Other negatives include doses of extracts assayed being too high, the positive control is not clearly defined or the method used unsuitable .The assay itself should be compatible with the type of extract or compound to be tested, for example it is not realistic to expect a chloroform or hexane extract to mix with, or diffuse through, an aqueous agar medium.

Conclusion: The fact that a plant extract exhibits activity is of interest, but it is only a preliminary piece of data that should be followed by the identification of the active compound(s) involved by means of a bio-guided assay. The research should be extended to uncover as much potentially interesting data as possible, including toxicity against animal or human cells, mechanisms of action, effects in vivo and positive or negative interactions with common antibiotics (Rios and Recio, 1995).

Keywords: pland extracts, antimicrobial compounds, bioassay techniques

O43: **Use of PK/PD principles to optimize and safeguard antibacterial therapy**.

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Aims:
The aim of this presentation is to provide knowledge about the use of PK/PD-principles to safeguard and to optimize antibacterial therapy; therapy of respiratory tract infections is used as an example.

Summary:
Respiratory tract infections (RTIs) continue to be a major source of morbidity and mortality. Attainment of pharmacokinetic/pharmacodynamic (PK/PD) targets is one of the relevant factors determining therapeutic outcome of an infection. Furthermore, PK/PD calculations can be used to safeguard therapy. Typically, PK/PD assessments are based on the susceptibility, i.e. the minimal inhibitory concentration (MIC) of the agent for the pathogen, and preclinical infection models. In vitro-, ex vivo- and in vivo infection models as well as in vitro pharmacokinetic models and in silico modelling have been developed to explore antibacterial pharmacodynamics. Any of the models used are indented simplifications of the clinical situation. This information is supplemented with pharmacokinetic data like the maximal serum concentration (Cmax) or area under the serum concentration versus time curve (AUC) of the free fraction of the drug in serum. Thus, PD-characteristics can be correlated with PK-parameters.

However, the PD-parameter, i.e. the susceptibility (MIC) pattern of a given pathogen as well as the PK-parameters of a given agent are not single point estimates but provide a Gaussian distribution, so that the possible combinations of kinetic and dynamic values are extremely complex. The use of probabilistic methods factors in the entire range of possible surrogate values and the probability of achieving them. Therefore, probabilistic methods should be used for PK/PD calculations to safeguard therapy of RTIs and to predict the likelyhood of clinical efficacy of an antibacterial agent. As both, the susceptibility pattern of each and every species of pathogens causing a RTI, as well as PK in different patient populations differ, population-based approaches differentiating between the various bacterial species as well as defined groups of infected patients (e.g. CAP, HAP, VAP, etc.) should be used for PK/PD considerations. For example, the resistance rates (and thus the MICs inhibiting 90% of the isolates) P. aeruginosa strains isolated from cystic fibrosis patients are as high as 54%, strains isolated from VAP-patients having been treated previously with a quinolone were found to be 57% resistant, whereas those isolated from non-treated VAP-patients were 26% resistant, and the isolates from HAP-patients were 18% resistant. Consequently, it is important to base PK/PD calulations on strain populations having been isolated from patients suffering from a specific infectious disease. Generalizing calcualtions based on a given species irrespective from the origin of the material (i.e. infectious site) from which the strain has been isolated, are misleading. By applying the differentiating approach it becomes evident that first, MICs of the pathogens change over time; emerging resistance passes unnoticed in routine susceptibility testing but puts the patient at risk. PK/PD calculations detect susceptibility shifts early on, thus safeguarding antibacterial therapy. Based on PK/PD calculations, either the dose should be adjusted, or the authorities should change the usage categorization precluding the empirical use of agents. Once granted marketing authorizations should be revisited every two to five years based on the actual MIC-distribution and probabilistic PK/PD calculations. Second, PK of an agent differ not only between healthy volunteers and patients, but also between different patient-populations.Third, PK (in particular their penetration into different infectious sites) of various agents even of one drug-class differ. Thus, PK/PD surrogates, too, differ indication-, species-, and drug- specifically. Consequently, PK/PD surrogates used to safeguard therapy should not be defined class-specifically but should be defined for a specific agent causing a specific disease. In conclusion, consideration of disease- and species-specific factors in PK/PD calculations will optimize the likelyhood of therapeutic success.

O44: **Resistance surveillance studies as a tool to control resistance - problems and pitfalls.**

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Aims: The aim of this presentation is to demonstrate that on the one hand surveillance studies are early warning systems indicating the emergence of resistance. On the other hand, however, surveillance studies may be misleading as resistance rates are highly variable.

Summary: It is well documented that surveillance studies generate data which vary internationally, nationally, regionally, and even within one hospital from ward to ward. Because of the highly variable resistance rates, the following questions can be discussed: 1. at which threshold of resistance should an agent no longer be used empirically, 2. does local variability allow a generalizing nationwide recommendation to refraim from using a given drug in a specific drug/bug association, 3. is the key denominator for result interpretation the clinical source of the isolate and patient condition of just the bacterial species as such, 4. are datasets biased, as isolates from hospitalized and/or difficult to treat pateints may be over-represented, 5. who is the user of the data?

Ad 1) Most frequently, authorities and societies use a resistance-threshold-level of 10% above which an agent should no longer be used empirically. Threshold-levels of 20 or even 30% are used as well. However, such threshold are not linked to clinical outcome. By linking the prevalence of e.g. macrolide resistance to clinical outcome it was demonstrated that the pre-defined threshold of 10% was inadequate and resulted in treatment failures and deaths. Therefore, threshold definitions should not only consider resistance statistics, but also clinical outcome. Ad 2) Restricted geographical sampling for limited periods of time results in point-prevalence studies, but not in representative surveillance data. Such studies provide information about the local resistance frequency, which is important information for the treating physiciam, but not information about the resistance epidemiology, prevalence, or incidence. Ad 3) The choice of sampling methods and organisms as well as the selection of the host population to be sampled has a fundamental impact on the outcome of surveillance studies. It is essential not to collect in very general terms species-specific laboratory- based surveillance data, but, instead, to use infection-based information stratified according to the severity of disease, patient population, and risk profile. Ad 4) Many agents are most frequently prescribed by general practitioners to outpatients. However, outpatients are sampled only when initial therapy has failed and in whom resistant subpopulations will likely have been selected. Selective testing in difficult to treat patients or a situation of treatment failure does not reflect the resistance epidemiology in the majority of outpatients. Furthermore, ignoring intercenter variation and differences in the numbers of samples collected per center leads to erroneous conclusions about resistance frequencies. Ad 5) The requirements of the users are often not met; the prescribing physician, the microbiologist, the infection control specialist, public health and regulatory authorities, the politician, and the pharmaceutical industry have diverse interests, which, however, are not addressed by different designs of a surveillance study. Tools should be developed to provide customer-specific datasets.

Consequently, most surveillance studies suffer from well recognized but uncorrected biases or inaccuracies. Nevertheless, they provide important information that allows the identification of trends in pathogen incidence and antimicrobial resistance.

O45: In silico identification of potential therapeutic targets for new vaccine design and Biological adjuvants

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Availability of genome sequences of pathogens has provided a tremendous amount of information that can be useful in drug target and vaccine target identification. One of the recently adopted strategies is based on a subtractive genomics approach, in which the subtraction dataset between the host and pathogen genome provides information for a set of genes that are likely to be essential to the pathogen but absent in the host. The availability of genome-scale sequenced data of more than 60 microbes in the past decade and the completion of the human genome project has revolutionised the field of drug- discovery against threatening human pathogens. The strategies for drug design and development are progressively shifting from the genetic approach to the genomic approach. Novel drug targets are required in order to design new defence against antibiotic sensitive pathogens. Comparative genomics and bioinformatics provide new opportunities for finding optimal targets. The entire approach is built on the assumption that the potential target must play an essential role in the pathogen's survival and at the same time, this target should not have any well-conserved homolog in the human host.

In our laboratory with the help of in silico studies we evaluate various virulence factors of E.coli strains for vaccine design or their application as biological adjuvants.

O46: **The intestinal Microbiota and its interactions with the host**

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Healthy adult harbors more than 100 trillion bacteria in gut alone, which is 10 times more than human cells. Human also posses 23000 genes, while its microbiome contributes 3300000 ones, mean that microbial genome is 150 times larger than human genome. Many of the trillions of microbes that live in the human intestine have been characterized and categorized, but still more bacteria have not been identified and cultured yet. Now we do understand that bacteria can occupy our bodies and live in concert with us as their human host. They need to either supply us with something that is beneficial or they need to support other bacteria that live in or on our bodies to enable their existence and selection to coexist with us. There are modern sets of assumptions that these organisms are living in a mutualistic relationship with us, and on that basis we assume that they're providing a health benefit, including prevention of colonization of bacterial pathogens, education of the human immune system, and also presenting metabolic role, such as providing salvage calories, improving nutrient absorption, production of short chain fatty acids, arginine, glutamine, vitamins, and acid folic. They also participate in drug metabolism and deconjucation of bile acids, brain function and overall health.

The gastrointestinal microbiota changes throughout the life. Many elements of the modern lifestyle have been postulated to result in changes in the gut microbiota, including dietary changes which not only alters the composition of the human gut microbiota but also provides substrates for the production of small molecules that influence disease development. Indeed, many aspects of our environment have been changed dramatically over the past few decades concurrent with the increasing incidence of gastrointestinal tract diseases. Scientists are working in a variety of settings to try to understand how these microbes can affect and influence normal and abnormal human conditions, ranging from changes in body weight to cancer. These scientists are looking for ways to use this information to improve human health as well.

Intestinal microbiota has well established role in enteric infections and infestations, including gastritis and cancer, antibiotic associated diarrhea, and small intestinal bacteria overgrowth. This role is also postulated for disorders such as fatty liver disease, obesity and metabolic syndrome, diverticulitis, inflammatory bowel disease (UC, CD), irritable bowel disease, celiac disease and etc. Association studies more recently revealed that humans who were in health state had certain species of bacteria that were more abundant or more prevalent than in those who were in a disease state. The study of obesity and the relationship with the gut microbiome showed us this differences, but the question is that how we can attribute such relationships with particular types of diseases? Researchers recently found the fact that feeding of livestock with low doses of antibiotics in the agricultural industry for more than 50 years has effect on increasing the body mass. This association was established in an experiment in a mice model such that group of mice under feeding with low doses of antibiotics developed an increased fat percentage in their body as a result of the change in the gut bacterial population. Accordingly, it seems plausible that gut microbiota can affect human health and influence its metabolism and body composition. Obesity, diabetes, metabolic syndrome, nonalcoholic fatty liver disease and cancer are among these hypothesized disorders. Beyond the direct role of gut microbiota in the human diseases, metabolic products of colonic bacteria, including ethanol and other volatile organic compounds, may also have toxic effects on human health after intestinal absorption and delivery to the liver via portal vein. Changes of gut microbiota may cure such bowel disorders. New efforts on fecal microbiome transplantation for patients with recurrent Clostridium difficile infection showed noteworthy results for their treatment.

Increase of our knowledge about role of gut microbiota in human health and their associations with disorders, such as Crohn disease, inflammatory bowel disease, irritable bowel disease, obesity, and fatty liver disease will provides practical recommendations for designing new treatment strategies against them in future.

O47: New targets for antibiotics to combat antibiotic resistance

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Infectious diseases are a major cause of morbidity and mortality. Each year over 2 million people die worldwide as a consequence of bacterial infections notwithstanding the availability of antibiotics for more than sixty years. A major problem is that the number of bacteria that acquires resistance to the available antibiotics is emerging. Reasons therefore include the enormous adaptability of bacteria and the uncontrolled and improper use of antibiotics. An additional problem is that during recent years only very few new antibiotics reached the market and research for new antibiotics in major pharmaceutical companies is very limited. To overcome the problem of antibiotic resistance, besides a more rational use of antibiotics, there is an urgent need to look for antibiotics with a new mode of action based on novel antibiotic targets as such avoiding or diminishing possible cross resistance with existing antibiotics.

Based on bacterial genome sequence analysis, theoretically circa 300 essential proteins could serve as potential target. However, an ideal target should meet the following criteria: essential and conserved in bacteria; substantially different from the human counterparts and easily accessible for the drug. Focusing on the protein secretion pathway, which is very important for cell viability and functionality, two proteins of this pathway fulfill these criteria. Circa 30 % of the total bacterial proteome is secreted, making use of the general secretion pathway, also named Sec pathway. Proteins secreted via this pathway are produced in the cell as preproteins containing a “secretion tag”, the signal peptide, an N-terminal extension at the mature protein. On secretion, the preprotein enters the secretion pathway and is transported to the secretion translocon, which consists of three integral membrane proteins SecY, SecE and SecG, together forming the protein conducting channel. The peripherally associated motor protein SecA drives the preprotein through the channel by repeated cycles of ATP binding and hydrolysis. During or shortly after the transport across the Sec translocon, a membrane anchored signal peptidase will cleave off the signal peptide, and the protein will then be released from the membrane, either in the periplasm (Gram-negative bacteria) or outside the cell (Gram-positive bacteria). Both the signal peptidase and SecA are essential in the secretion process, and hence, for bacterial viability. As a consequence, when the function of the signal peptidase or SecA is blocked the cell will die, because proteins cannot be secreted anymore.

Details will be given about the antibiotic resistance problem, and the bacterial secretion pathway and possible screening methods to find signal peptidase and SecA inhibitors will be discussed.

O48: Is stool culture necessary for management and surveillance of Shiga-toxin producing E. coli (STEC) infection?

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Shiga toxin (Stx) producing Escherichia coli (STEC) were first described as Vero toxin producing E. coli (VTEC) leading to bloody diarrhea (hemorrhagic colitis) and the haemolytic uremic syndrome (HUS) about 30 years ago. The natural reservoirs of STEC are ruminant animals, especially cattle and only a subset of STEC, designated as enterohemorrhagic E. coli (EHEC) is able to cause severe disease in humans. Transmission of STEC to humans usually occurs via contaminated food or water and numerous outbreaks as well as sporadic cases of STEC infections and HUS have been documented worldwide. STEC strains carry phages encoding Shigatoxins 1 and/or 2. For both Stx 1 and Stx 2 several allelic variants are described. Most of EHEC strains possess the eae gene of enteropathogenic E. coli as an additional virulence factor conferring adherence to the intestinal mucosa. STEC not harbouring eae were long believed to be less virulent, but have also been shown to be the causative agent of STEC outbreaks as observed in Germany in May and June 2011. E. coli can be subclassified by O an H serotyping. STEC usually belong to a relatively limited number of O: H serotypes and can be classified in different seropathovars according to the risk of severe disease or association with outbreaks. The seropathovars A O157: H7 and O157: NM were documented in the vast majority of HUS cases. However, in some geographic regions, including Germany, non-O157 serotypes have been reported to account for up to half of HUS cases.

The cultural diagnosis of STEC infection is difficult. STEC must be differentiated from non-pathogenic E. coli in the stool flora of patients. Most strains of relevant seropathovars can be distinguished from other E. coli strains by the resistance against tellurite. However, sensitivity and specificity of selective media is still low for some individual STEC strains. Therefore, detection of Shiga toxin in culture supernatants or detection of stx genes using nucleic acid amplification techniques is common in routine diagnostic procedures for STEC disease. These techniques allow accelerated detection of STEC strains. However, the differentiation of EHEC from STEC with low pathogenicity in humans cannot be achieved. Next generation sequencing techniques might resolve the problems in EHEC/STEC diagnosis in the future.

O49: Investigation of protein aggregation in Gram-negative bacteria and its potential application as a novel antimicrobial strategy

Mohammad Shahrooei

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Background:

Today we are faced with a situation in which almost all antibiotics in use have been matched by resistant bacteria. This growing phenomenon of bacterial resistance, especially among Gram-negative bacteria, is now threatening to take us back to a pre-antibiotic era and necessitates an urgent search for new antibiotics or new anti-infective strategies to overcome antimicrobial resistance. Protein aggregation has gained remarkable scientific interest in recent years, due to its cellular toxicity in human diseases such as Alzheimer's and Parkinson's diseases. As deposition of protein aggregates is associated with cellular dysfunction and death in human disease, it has been suggested that the induction of protein aggregation in bacteria can be used as a new strategy to develop antibacterial agents/peptides.

Methods:

Using an algorithm for prediction of aggregation-prone motives in unfolded polypeptide chains, called TANGO designed by the Switch laboratory of VIB at the KU Leuven in Belgium, the aggregation-prone motives in the whole genome sequence of the Gram-negative Escherichia coli have been identified and as short (12aa) peptides were designed and synthesized. All peptides were initially screened against a range of representative Gram-negative bacteria at a single concentration of 25µg/ml using broth microdilution assay according to EUCAST recommendations and the MIC of active peptides was subsequently determined. The mechanism of action of active peptides was investigated using Electron microscopy techniques and molecular methods such as induction of susceptibility of resistant strains to aggregating peptides by cloning and expression of the target sequences in the resistant strains. The cytotoxicity and haemolytic activity of active peptides were investigated in vitro and in vivo.

Results:

At the concentration of 25µg/ml, 42 out of 124 designed peptides were active against E. coli strain. Of these 42 peptides, 12 were active against Klebsiellapneumoniae. One peptide was active against 2 other Gram-negative species (P. aeruginiosa and Enterobacter cloacae). Expression of targets of two inactive peptides in Enterobacter cloacae converted this strain from resistant to susceptible strain. Active peptides were bacteriocidal rather than bacteriostatic and their time killing kinetics were in the same way. No haemolytic activity for any of active but a little toxicity was observed for some of them.

Conclusion:

By performing this work, we expect to reveal the mechanism of protein aggregation in Gram-negative bacteria and evaluate the potential application of targeted protein aggregation as a new strategy to combat Gram-negative infections and overcome the antibacterial resistance in Gram-negative bacteria.

P1: **Pharmacologicalmechanismsofbiofilm formation by Pseudomonas. auroginosa Compare Baansolutionagainstdrugand Non-pharmacologic**

Safiyeh Abbasi[1]

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Background and Aim: Background and Objectives: Pathogenic bacteria Pseudomonas aeruginosa is an important opportunistic. Mucoid strains of Pseudomonas aeruginosa alginate is composed of substances that are very mucoid and has the most important role in biofilm formation. In this study the effect of antimicrobial agents on biofilm in pseudomonas terms of chamomile essential oil aeruginosa has been studied.

Methods: Tested methods: Biofilm formation in Pseudomonas aeruginosa strains were used as the M8821. Effect Chamomile essential oil antimicrobial disc diffusion method was used at a concentration of 50% DMSO long. Oil effects on biofilm formation in LB medium containing various concentrations of 4% and 22%, 7% from oil micrograms per ml and then incubated for 24 hours at 37 was measured by Fonseca. The medium was inoculated without essential oil as a positive control medium not inoculated without essential oil was used as a negative control. the biofilm ofPseudomonasaeruginosa in microplate biofilm formation was made of polystyrene. The effectiveness of conventional antimicrobial agents such as antibiotics penicillin, erythromycin, tetracycline and ,chlorhexidineof the biofilm on the number of viable cells was evaluated by MIC material tested was determined for Pseudomonas aeruginosa. Most bactericidal effects on biofilm after 5 min treatment with 2% chlorhexidine mg / ml% 9MIC and with minimal impact on tetracycline mg / ml MIC% 3 was observed. Clinical isolates of the biofilm OD OD biofilm biocide containing the same strain (ODr) showed that the thickness of the biofilm formed in the presence of effective microbial agents is low. Steady decline in the number of viable cells in the biofilm treatments erythromycin and penicillin were determined.

Results: Results: The bacteria had no antimicrobial essential oils, essential oil concentration in the presence of 4 ?g / ml and 22?g/ml biofilm formation was significantly higher than the positive control, but 7?g/ml biofilm formation in the presence of oil, significantly lower than the positive control. the survey also showed that eradication of biofilm viable cell concentration than drug treatment 5MIC antimicrobial substances is necessary.

Conclusion: Conclusion: Although chamomile essential oil has antibacterial effect on Pseudomonas aeruginosa, but is reduced biofilm formation. 5MIC drug concentrations higher than is necessary to prevent the formation of biofilms

Keywords: Pseudomonas aeruginosabiofilms, MC, penicillin, erythromycin, tetracycline, chlorhexidine

P2: **The effect ofsilver gram-negative pathogens on a wide range Bta-laktamaz by enzymes (ESBLs)**

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Background and Aim: Background and Objectives: One of the major problems of disease bacteria resistance Hospital to material anti Microbes is causing the problem of increasing health care costs, improve health finally dying patients. The purpose of this paper is to identify pathogenic gram-negative bacilli resistant to beta-lactam antibiotics terms of effect of Nano silver in them.

Methods: Methods: in culture of 276 clinical specimens of patients admitted to three hospitals) Gharazi, Sina and al-zahra in Isfahan isolated in 8 months of 1390 a total of 186 gram-negative bacilli's. These bacteria studies to produce extract spectrum beta -lactamase (ESBLs) by the disk diffusion method under study were tested using approved methods (Double Disk approximation Test, DDT) was performed. Bacilli producing ESBLs under effect of 400, 500 and 200,25,50,100 ppm12 / 5 Silver Nanoparticles prepared bark of Nano nasb Tehran company and the halo diameter none-growth was measured

Results: Results: 140 cases of ESBLs-producing bacillus Gram-negative and 46 patients (24\ 7%) were gram-negative, non-ESBL. Most of the ESBL infected with gram-negative samples isolated were urine sample and prevalent bacteria were K.pneumoniae. All of the samples soluble were susceptible to silver nanoparticles with 100 ppm concentration. Entrobacter. aurogenius(24 mm) and psedumonas.auroginosea(23 mm)the inhibition zone with presence of 500ppm silver nanoparticles concentration.

Conclusion: The results: Obtained show the Nanoparticles silver be can inhibitor effect on all of the gram-negative bacilli had tested and high concentrations of silver nanoparticles, the growth of gram-negative bacilli ESBL was increased. the Nano particular can be replace to anti-biotic but worthy of attention the resistance of clinical strain higher than the standard strain.

Keywords: gram-negative, ESBL silver nanoparticle

P3: **Contamination of radiographic cassettes in a trauma center; needs for system digitizing**

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Background and Aim: Radiography plays a vital role in medical management of trauma patients. Many studies have demonstrated that the radiology departments frequently becomes contaminated with pathogenic microorganisms and radiography equipment including cassettes, markers and beds are the main sources of pathogen and infection agents. The main aim of present study was to assess the level of contamination in radiographic cassettes in the radiology department in a trauma center.

Methods: The whole surface areas of all cassettes from all sizes in radiology department including 40 plastic and 10 aluminum surface based cassettes were swabbed in total specifically for general levels of bacterial and fungal contamination. Swabbing was performed twice, once to determine the extent of contamination of bacteria and in second for fungal contamination detection. The swabs were moistened and sterilized with distilled water to providing suitable conditions and keep organisms alive from the time of collection to the time it gets to the mycology laboratory. The prepared samples were immediately plated on to Cystine Lactose Electrolyte Deficient Agar plates (CLED) and blood agar media for culturing and detecting bacterial infections. They were then incubated at 37 C for 48 h. For fungal detection, sabouraud dextrose contained Chloramphenicol (SC) media was used and they incubated at 30 C for 1 week. After incubation, the plates were read and present bacteria were recorded as the number of colony forming units and also cultures were isolated and identified using standardized bacterial methods.

Results: Our result showed from 50 radiography cassettes that were swabbed, 47 (94%) cassettes were contaminated with bacteria and there were not any fungal spores in cassettes. The most common observed bacteria were Staphylococcus epidermis, Staphylococcus aureus and Diphteroids. The levels of contamination in plastic cassettes were more than aluminum cassettes. Among all cassettes, the highest level of bacteria was found on a 30*40 cassettes.

Conclusion: This report has demonstrated that radiographic cassettes in a busy trauma center are main sources of pathogenic agents such as bacteria and they can distribute nosocomial infections in all parts of hospital. In this busy center, there is no enough time to clean cassettes and trauma patients skins has a near contact to radiographic cassettes frequently and their health are at risk. Our best recommendation for solving this problem is digitizing the radiology center and removing cassette-film system.

Keywords: Trauma center, Radiographic cassettes, Nosocomial infetions,

P4: **The Application of Probiotics in Radiotherapy: An Idea Based on Genetic Manipulation of Probiotics**

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Background and Aim: In recent years many attempts have been made to spare organs at risk from radiation fields (physical methods) or protect them by pharmaceutical and natural agents (chemical methods). Advances in dose delivery techniques, image guidance therapy, targeted therapy , radioprotectors and mitigators are developed methods to reduce normal tissue injuries. Finding radioprotectors/mitigators with lower systematic toxicity and highest efficiency also is one of the main goals of radiation researchers. Probiotics are live microorganisms that have health benefits. According to the World Health Organization (WHO) probiotics are 'live microorganisms which, when administered in adequate amounts, confer a health benefit on the host'.

Methods: In this letter, we suggest and hypothesized that probiotics can be used for normal tissues protection during or after radiotherapy.

Results: In addition to trophic effects on the gastrointestinal tract, probiotics have much potential include toxin neutralization, antagonistic activity, synergistic activity and stimulation of immune system [4]. Preventive effects on the induction of nuclear factor-kappa B (NF-κB), expression of tumor necrosis factor-α (TNF-α) and other pro-inflammatory cytokines, production of anti-oxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) and therefore free radical scavenging underlie those potentials [5]. In regard to these preventive and stimulative effects, I proposed to use probiotics as a feasible and continuous regimen during or after radiotherapy. The radical scavenging and immunostimulation trait of probiotics makes it as an appropriate radioprotectors in radiotherapy. In the other hand, probiotics are usually safe but sometimes can cause complications and side effects. The other important side of this idea is genetic manipulation of probiotics to make them more beneficial as radioprotectors. Genetic improvement of probiotics for developing suitable radioprotectors with lower toxicity, lower side effects, more radical scavenging and more immunostimulatory effects can be done in future to enhance radiotherapy efficiency. The secondary cancer preventive role of probiotics in this area can be important particularly in children and other radiosensitive peoples.

Conclusion: By genetic manipulations of robiotics, they can be feasible radioprotectors in radiotherapy.

Keywords: Probiotics, Radiotherapy, Normal tissue protection, Genetic manipulation

P5: **Impact of Nanoparticles on bacterial load reduction of contaminated wounds in mice**

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Background and Aim: Wound site infections have become a problem for patients and health services. The microorganisms have been usual noted as the cause of delayed wound healing. The most common pathogen causing these infectionsis Pseudomonas aeruginosa. Nanoparticles in nanomedicine are defined as particulate dispersions or solid particles with a size in the range of 10-100 nm. Silver nanoparticles (AgNPs) show ample antibacterial activities, which confer to them a major advantage for the development of alternative products; AgNPs are effective against pseudomonas aeruginosa in vitro. In present study, the effect of AgNPs alone and in combination with tetracycline investigated on inoculated wounds with Pseudomonas aeruginosa PAO1 in mice.

Methods: Twenty mice were randomly divided into four equal groups (n=5); All mice were anesthetized and full-thickness skin wounds were created on the back of mice and the bacterial suspension (106 CFU/ml) was added to each wound bed. In all of the groups treatments applied topically in the wound bed: AgNPs, tetracycline, AgNPs along with tetracycline and saline normal in control group.Wound infection assessed on 0, 4, 8 and 12 days post wounding.

Results: At day 8, the surface infection evaluation showed significant decrease in bacteria counts ($p < 0.05$) for treatment groups with AgNPs, tetracycline and AgNPs along with tetracycline (13×104 CFU/10µl - 11×104 CFU/10µl - 6×104 CFU/10µl) compared with controls (31×108 CFU/10µl). In all of the treated groups with AgNPs, tetracycline and AgNPs along with tetracycline showed decreases in surface bacterial concentration (0 CFU/gr) compared with control group (60 CFU/gr). Also, significant decrease ($P<0.001$) in deep skin bacterial counts in the AgNPs, tetracycline and AgNPs along with tetracycline were found at any time point compared with control group.

Conclusion: Application of AgNPs along with tetracycline is more effective than AgNPs and tetracycline alone to reduce the microbial load and wound macroscopic contraction. These findings support use of the AgNPs in combination with antibacterial medicine for the treatment of skin wounds.

Keywords: Wound infection, Pseudomonas aeroginosa, Silver nanoparticles

P6: **Frequency of Neisseria gonorrhoeae endocervical infection among female patients and Changing trends of Antimicrobial Susceptibility patterns in Kashan, Iran**

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Background and Aim: Background: Neisseria gonorrhoeae is one of the most general sexually transmitted diseases in developing countries, and the emergence of resistance to antimicrobial agents in gonococci is a chief problem in the control of gonorrhea. The aim of this study was determination of endocervical gonococcal infection and antibiotic susceptibility in Kashan, Iran. Aim: The present study emphasizes the importance of surveillance of antimicrobial resistance of N. gonorrhoeae in order to supervise the rate of multi resistant strains and to revise the cure recommendations.

Methods:: In this descriptive study, 294 endocervical swabs were collected from the obstetrics and gynecology clinics between December 2012 to May 2013 of the Kashan, Iran. The samples were cultured in modified Thayer Martin in 37° C with 5% CO2 for 48 h. Gram staining, oxidase, catalase and carbohydrate utilization test were used to confirm the isolated species. All the isolates were tested for antimicrobial susceptibility using the Kirby Bauer-disc diffusion techniques.

Results: The overall risk of gonorrhea was 2.38% (95% confidence interval [CI] 1.5-3.26%). All isolates were resistance to ceftriaxone, penicillin G, ciprofloxacin, tetracycline, cefepime, except for 2 isolate that was intermediate to tetracycline

Conclusion: The prevalence of N. gonorrhea infection among female adolescents is relatively high. These findings support early and comprehensive sex education and N. gonorrhea screening of sexually active female adolescents. N. gonorrhea has developed significant rates of resistance to various antibiotics and resistant N. gonorrhea strains are increasing and high-level resistant strains are also present in Kashan, Iran.

Keywords: Neisseria gonorrhoeae, antimicrobial resistance, endocervix

P7: **Evaluation the prevalence of extended-spectrume betalactamaz type 1 at acinetobacter isolations from clinical specimen at educational hospitals of sari city 1392**

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Background and Aim: Prevalence of class A extended-spectrum -lactamases (ESBLs) has been investigated in Acinetobacter baumannii. The aims of this study were to determine the prevalence of class A ESBL-producing A. baumannii and to characterize the genotypes.

Methods: During the period of January to June 2013, clinical isolates of A. baumannii was collected from Burn patients in zare Hospital, in sari city . Antimicrobial susceptibility was determined by the disk diffusion and Etest methods, and ESBLproduction by the double-disk synergy test. Searches for blaTEM, blaSHV, blaCTX-M, blaPER-1, blaVEB, and blaGES/IBC genes were performed by PCR amplification, and the genotypes of ESBLs were determined by a direct nucleotide sequence analysis of the amplified products.

Results: A total of 60 clinical isolates of A. baumannii seven (11.66%) isolates of A. baumannii isolates showed positive results in the double-disk synergy test using ceftazidime and ceftazidime –clavlunic acid disks, and cefotaxime , cefotaxime-clavlunic acid. The most prevalent class A ESBL genotype in A. baumannii isolates was blaPER-1 (n=7).

Conclusion: It is concluded that class A PER-1 ESBL-producing A. baumannii isolates are spreading,has emerged in this hospital . The spread of class A ESBLs could compromise the future usefulness of expanded-spectrum -lactam antibiotics for the treatment of A. baumannii infections.

Keywords: Acinetobacter baumannii, Class A ESBL- sari

P8: **Fed-batch fermentation of lactic acid by use of molasses**

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Background and Aim: Lactic acid is a raw material for the synthesis of polylactic acid (PLA) that is a useful biodegradable plastic material. However, the high cost of lactic acid production has been the bottleneck for practical applications. Molasses which is a by-product of sugar refining plant widely used as a raw material for fermentation, due to the abundance and low cost can reduce the production cost. The aim of this study is to produce lactic acid from molasses as a carbon sources by fed-batch fermentation.

Methods: The batch fermentation model was performed in 3 L fermentor with basal medium and molasses as a carbon source and feeding with lactose was started after 36 hours for 8 hours by constant speed of 0.03 L/h. Fermentation was carried out by use of Propionibacteriem freudenreichii ssp. shermanii and Lactobacillus acidophilus as a co-culture at 37 ° C and pH=6.5 for 144 h. Samples of 20 ml were removed each 24 h of the fermentation. Content of biomass and organic acids were measured by freeze drying method and HPLC, respectively.

Results: The final concentration of the dependent variables obtained as following (grams per liter): biomass 1.35± 0.05, lactic acid 28.42 ± 0.06, propionic acid 0.48± 0.01 and acetic acid was not detected. Also, vitamin B12 (~ 3 mg/L) was detected in this study.

Conclusion: This study is considered to provide a low-cost process for fed-batch production of lactic acid from cheap resources and also it can be an interesting alternative method for microbial production of vitamin B12.

Keywords: Lactic acid, fed-batch fermentation, molasses

P9: **Comparison of tip-alpha gene expression of Helicobacter pylori in strains isolated from patients with Gastritis and Gastric Cancer with use Real Time RT PCR**

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Background and Aim: In recent years, a group of genes that encode proteins associated with the pathogenesis of H.pylori are found be associated with cancer. Tip-α protein increases activation of Nuclear Factor Kappa-B (NF-KB) caused to more expression of genes IL-1, IL-8, TNF-α in host cells. Some studies show different of tip-α gene expression in isolated strains of H. pylori from Gastric cancer and NUD biopsies. The aim of this study was to evaluate tip-α gene expression in Helicobacter pylori strains isolated from Iranian patients with Non Ulcer Dyspepsia (NUD) and Gastric Cancer (GC).

Methods: Seventy five samples from patients with NUD as calibrator (25 patients, mean age of 48) and 75 specimens from patients with gastric cancer as subject (25 patients, mean age 61 years) were collected. Total RNA was extracted by Tripure reagent according to the manufacturer protocol. cDNA was synthesized from total RNA by Quantitect Reverse Transcription, according to manufacturer instructions. Primers were designed for Primary-Single PCR and Real-Time PCR by the Primer3 software then were blasted with online NCBI blast program A primary Single-PCR was performed for increase of little amount of bacterial cDNA. Real-Time PCR was performed on diluted products of primary Single-PCR. Amplification reactions were performed on a Corbett Rotor-Gene 6000 Real-Time PCR System based on the SYBR Green methodology.

Results: The relative expression of tip-alpha gene in the GC group was found to be increased in 1.34 times in compare with NUD group (DeltaCt method). So no significant different in tip-alpha gene expression was founded between two groups ($P< 0.05$).

Conclusion: Its seem tip-α has no role in clinical outcomes singly. Although more research is need in this regard.

Keywords: tip-α ،Helicobacter pylori ،Real-Time RT PCR

P10: **Prevalence of Metallo-β-Lactamases-producing Klebsiella pneumoniae strains in patients referred to Hamadan university of Medical Sciences hospitals**

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Background and Aim: Klebsiella pneumoniae is one of the major causes of nosocomial infections particularly in patient with immunodeficiency. Several antibiotics used in treatment of infections with these bacteria such as Betalactams. Metallo β-Lactamases (MBLs) are bacterial enzymes with Extended-Spectrum activity against beta-lactams. Four groups of MBLs have been described up to now, namely, IMP, VIM, SPM and GIM. The IMP and the VIM types are prevalent and worldwildly. In addition, VIM1 and VIM2 are two subtypes of VIM and IMP1 is a subtype of IMP group. Aim of this study was Evaluation of MBL-producing isolates of Klebsiella pneumoniae resistant to Imipenem by PCR and DDST methods.

Methods: This research was performed on 100 Klebsiella Pneumonia strains that were collected from patients referred to Hamadan university of Medical Sciences hospitals during 2008-2010. The strains were distinguished separately with two microbiological and PCR methods. Detection of blavim1, blavim2 and blaimp1 genes was done by DDST method for phenotyping and PCR for genotyping. In addition, the antimicrobial susceptibility tested with Kirby-Bauer and Etest methods.

Results: According to the results, Imipenem-resistant strains were detected 12% and 8% with using Kirby-Bauer and Etest methods respectively. Furthermore evaluation of DDST method showed that 5% of these strains have Metallo-beta-Lactamase enzyme. Finally, evaluation of blavim1, blavim2, blaimp1 genes showed that 5% of strains have only blavim1 gene and none of them have blaimp1 and blavim2 genes.

Conclusion: The genotyping and phenotyping experiments are efficient for detection of antibiotic resistance. These results show high prevalence of blavim1 gene in Klebsiella Pneumonia strains isolated from patients referred to the hospitals of Hamadan university of Medical Sciences.

Keywords: Klebsiella Pneumonia, MBLs, DDST, Etest, PCR

P11: **Isolation and differential diagnosis of Vibrio cholerae O1 and O139 in Iranian Kurdistan border towns**

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Background and Aim: Vibrio cholerae is cause of the cholera disease and deaths in different parts of the world. Vibrio cholerae O1 and O139 biotypes have worldwide spread and are cause of epidemic cholera. In spite other classic strains are sporadic. In this study, due to overlap of biochemical tests results between strains of O1 and O139 biotypes with other strains, we designed a PCR protocol with using O1 and O139 biotypes specific genes primers for obtain a rapid method with high sensitivity and high specificity in detecting of cholera specimens. Another aim of this study was detecting of ompw, ctxa and tcpa genes.

Methods: 30 samples were collected from the border towns of Kurdistan during 2013 and were sent to Reference Laboratory in Kurdistan University of Medical sciences. Samples were assessed by biochemical tests initially. The primers genes ompw, ctxa and tcpa were used in PCR protocol.

Results: Biochemical tests results of 16 samples were positive. PCR results were shown 14 specimens had tcpa gene, 10 specimens had ompw gene and 5 specimens had ctxa gene.

Conclusion: The results of this study shows that PCR technique has not advantage over biochemical diagnostic methods in detecting of O1 and O139 biotypes significantly ($P< 0.05$).

Keywords: PCR , Vibrio cholerae , tcpa , ompw , ctxa

P12: **Antibacterial properties of Dendrimers**

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Background and Aim: Since drug-resistant bacteria have been increased, the research and discovery of new antibacterial agents is essential. Dendrimers (such as polypropylene amine: PPA) have specific properties that distinguish them from other antimicrobial agents. Dendrimers are branched and regular polymers that have a very active group against the bacterial cell wall and bacterial cell membrane. The aim of this study was survey of the PPA antimicrobial power with using several different techniques.

Methods: Bacterial strains used in this study were Escherichia coli (ATCC 8739) Staphylococcus aureus (ATCC 6538) and Bacillus subtilis (ATCC 6633). These strains transferred to nutrient broth and incubated in aerobic conditions for 24 h at 37 °C initially. The bacterial optimal concentration was prepared according to McFarland 0.5 standard. In order to evaluate the PPA antibacterial activity, serial dilutions (0.1, 0.01, 0.001, 0.0001, 0.00001,) were prepared in sterile saline. For evaluate the toxic effects of PPA against bacteria, determination of Zone of inhibition, determination of minimum inhibitory concentration (MIC) and determination of minimum bactericidal concentration (MBC) methods were used.

Results: The size of the inhibition zone in around of disks had a direction relationship with increasing of the PPA concentration. The Minimum concentration of PPA that prevents bacterial growth (MIC) and Minimum concentration of PPA that kills bacteria (MBC) for Escherichia coli, Staphylococcus aureus and Bacillus subtilis were 0.001 and 0.01 respectively.

Conclusion: According to the above results, a PPA antibacterial agent is effective against both Gram-positive bacteria and Gram-negative bacteria. The results show Gram-negative bacteria in compare to Gram-positive bacteria are more susceptible to the antibacterial agent of PPA.

Keywords: Dendrimers, Polypropylene amine, MIC, MBC

P13: **Detection of Shigella dysenteriae and E.coli O157: H7 toxins by Multiplex PCR method in clinical samples**

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Background and Aim: Bacteria producing shiga toxin (Shigella dysenteriae , E.coli O157: H7) associated with outbreaks of diarrhea, hemorrahagic colits, sever inflammatory in ileocolonic regions of gastro intestinal tract, thrombocytopenia, modulate physiological function of immunocytes, hemolytic uremic syndrome (HUS), Central nervous system(CNS) in humans. Most clinical sings of these diseases arise of producing shiga toxin (shiga toxin1, shiga toxin2) or combination of both types of these toxins. Since different methods diagnostic such as cell culture, Elisa, RFPLA, has been used to detection of shiga toxins. However, regard to having high cost, consuming a lot of time, having low sensitivity, has been less attention. In this study we used Multiplex PCR method for detection genes encoding shiga toxins which associated illness in humans.

Methods: Initial confirmation types bacteria producing shiga toxins used in this study were performed by biochemical and serological methods For detection of stx1 and stx2 genes two pair primers were designed which the Tm was near to each other and acting specifically for these genes. The fragment which obtained from stx1 primers was 490bp and for stx2 was 275bp, The PCR products were confirmed by sequencing. For specificity of this method, the bacteria Salmonella, Pseudomonas aeruginosa, Klebsiella pneumonia, Vibrio cholera was used as a negative control. The serial dilution of genome extraction was used as sensitivity method.

Results: PCR amplification products identifying the stx1 and stx2 gene sequences were observed only in E. coli O157: H7 and shigella dysenteriae. These primers were not cross reaction with other gram-negative bacteria.

Conclusion: This method is fast and careful for detection bacteria producing shiga toxin and can be used to identify types of shiga toxin.

Keywords: Stx, E.coli O157: H7, Shigella dysenteriae

P14: **Designing Novel and Simple Competitive Internal Amplification Control for Reliable PCR Diagnosis of HSV Keratitis and Encephalitis**

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Background and Aim: In this study, design of competitive internal amplification control for PCR diagnosis of herpes simplex virus (HSV), that is the main cause of keratitis and viral encephalitis in developed countries, is novel and reported for the first time. PCR is a molecular technique for herpes simplex virus (HSV) detection, which can cause life-threatening infections such as encephalitis and keratitis. However, false-negative resultsts, causing by PCR inhibitors, are the main issue of this technique that reduces the PCR efficiency. To overcome the mentioned problem, competitive internal amplification control (IAC) was constructed for conventional PCR using PCR-cloning technique.

Methods: Composite primers for PCR amplification of Leishmania major kDNA (kinetoplast DNA) were designed and optimized to use as IAC-HSV. IAC-HSV amplified in a non-stringent condition; ligated into pTZ57R plasmid vector, transformed into E. coli JM107 and then cloned. Resulting IAC was used for 105 CSF and 78 keratitis specimens.

Results: PCR amplicons for HSV and IAC-HSV were 454-bp and 662-bp, respectively. Detection limit of IAC was determined as 1,000 plasmids per PCR reaction. IAC sensitivity for HSV detection was 500 copies/mL of HSV DNA. Among all specimens, 7 inhibited specimens were detected.

Conclusion: Conclusion: Indeed, using another DNA as an internal amplification control is expected to detect false-negative results and amplification of this DNA is the key tool to examine the accuracy of amplification and detection steps. This internal amplification control is applicable for early reliable diagnosis of HSV in different loads of virus in different specimens.

Keywords: herpes simplex virus, PCR, Leishmania major, pTZ57R, internal amplification control, false-negative result.

P15: **Antibiotic resistance pattern of ESBL and non ESBL producing P.aeruginosa isolates by micro broth dilution**

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Background and Aim: Pseudomonas aeruginosa as a opportunistic pathogen is one of the most prevalent bacteria in nosocomial infections. This organisms has resistance to many useful antibiotics including extended spectrum beta lactams . in this study antibiotic resistance patterns for clinical useful antibiotics agains ESBL and non ESBL producing P.aeruginosa isolates were detected by micro broth dilution method.

Methods: in this study 147 clinical P.aeruginosa isolated from Milad hospital in Tehran during 8 months in 2012, were collected. Isolates were identified by standard biochemical methods and ESBL producing isolates detected by phenotypic methods including disk diffusion method as screening and double disk as confirmatory method. For final analysis 76 isolates including 50 ESBL producing and 26 non ESBL producing were selected. Minimum inhibitory concentration (MIC) for ceftazidim (CAZ), aztreonam (ATM) , piperacillin (PIP), carbenicillin (CN), ticarcillin (TIC), amikacin (AN), gentamicin (GM), meropenem (MER) and ciprofloxacin (CIP) (supplied from Sigma) against P.aeruginosa isolates by micro broth dilution in muller hinton medium (CA-MHB) according to CLSI guidelines were detected.

Results: Among 76 isolates, 58 (76.3%) were resistance to more than three antibiotic and identified as MDR strains. Rate of resistance to ceftazidim, piperacillin, ticarcillin, carbenicillin, aztreonam, meropenem, gentamycine, amikacin and ciprofloxacin were 84.2, 71, 77.6, 72.3, 88.1, 64.4, 76.3, 76.1, and 81.5%, respectively. Meropenem and amikacin had the most activity against ESBL and non ESBL producing isolates, respectively.

Conclusion: According to the results, high rate of resistance to beta lactam antibiotics and also MDR isolates among P.aeruginosa isolates is as a major problem in treatment of infections caused by this organism. It should be stressed that using of standard diagnosis methods for detection of MDR and ESBL producing isolates specially in the hospitals are very essential and also many preventive methods for control of nosocomial infections and control of spread of MDR and ESBL producing isolates would be using in the hospital.

Keywords: Pseudomonas aeruginosa, Multi drug Resistance, ESBL, MIC

P16: **Determination of antibiotic sensitivity of Bacteroid fragilis isolated from patients and healthy individuals of teaching and treatment center of Imam Reza-Tabriz**

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Background and Aim: Background: Bacteroides fragilis are one of the most important anaerobic bacteria that play role in most of anaerobic infections. They have acquired resistance to essential antibiotics for treatment of anaerobic infections, more than other anaerobic bacteria. Objectives: The goal of this study is to determine the resistance of isolated Bacteroides fragilis against common antibiotics in the treatment of anaerobic infections.

Methods: A total of 188 fecal samples including 59 samples from hospitalized patients, 84 samples from outpatients, and 45 samples from healthy individuals were collected. The samples were cultured in Bacteroides- Bile- Esculine agar and Kanamycin-Vancomycin-Laked Blood media and were incubated in anaerobic atmosphere at 37oC for at least 48 hr. Suspected one mm colonies with a black surroundings, were selected and were identified using MID8 as well as biochemical tests. For MIC determination of antibiotics against isolated Bacteroides fragilis Etest was used.

Results: There was not any difference between antibiotic resistance patterns of isolated Bacteroides fragilis from hospitalized patients, outpatients, including diarrheal and non diarrheal cases, and resistant pattern of isolates from healthy individuals. All or most of isolated Bacteroides fragilis were susceptible to imipenem (100%), metronidazole (95%), rifampin (100%) and piperacillin/tazobactam (95%) and in contrast, to other antibiotics such as clindamycin (90%), and chloramphenicol (55%) showed resistance.

Conclusion: In the present study a number of important antibiotics in the treatment of anaerobic infections have lost partly or totally their positive effects on the Bacteroides fragilis.

Keywords: Bacteroides fragilis, antibiotic resistance, anaerobic infection, metronidazol, imipenem

P17: **Comparison of Etest and disk diffusion test for antibiotic resistance testing of enterotoxigenic and non- enterotoxigenic Bacteroid fragilis isolated from stools**

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Background and Aim: Enterotoxigenic Bacteroides fragilis (ETBF) are one of the most important anaerobic bacteria that cause diarrhea in human beings. A bft gene is coded for enterotoxin production called fragilysin. Most of them have acquired resistance to anti-anaerobic bacteria agent like other facultative anaerobic bacteria. Etest and different dilution methods are usually carried out for antimicrobial susceptibility determination of Bacteroid fragilis. Objectives: We aimed to recognize ETBF by PCR method and also to evaluate efficiency of disk diffusion method (DDM) in comparison with the Etests for antimicrobial susceptibility of B. fragilis isolates.

Methods: bft gene was detected among 157 Bacteroides fragilis isolated from patients and healthy individuals by PCR. Antimicrobial susceptibility of all isolates was determined by DDM and Etests methods.

Results: Nineteen (12.1%) B. fragilis containing bft gene from diarrheic (n= 14) and non-diarrheic (n=5) feces were detected among 157 B. fragilis isolates. The highest resistance for ciprofloxacin, cefotaxim, cefoxitin with DDM and Etest were 100%, 60% and 65% respectively, while the lowest resistance in two methods was obtained for imipenem, piperacillin/tazobactam, and metronidazol. 100% agreement for some of antibiotics such as imipenem ($P \leq 0.05$) and no correlation for others were observed among the antimicrobial susceptibility results obtained by two methods ($P \geq 0.05$).

Conclusion: The presence of bft gene in B. fragilis isolates will not certainly result in a diarrheal among patients. There is not enough accordance between DDM and Swedish Etests for antimicrobial susceptibility of B. fragilis for some antibiotics, although in other cases a good agreement was observed.

Keywords: Bacteroides fragilis, antibiotic resistance, disk diffusion method, Etest, bft gene.

P18: **The frequency of antibiotic resistance and the extended spectrum beta-lactamases(ESBL) in Escherichia coli isolated from urinary tract infections of out-patients in Kermanshah, Iran**

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Background and Aim: urinary tract infection (UTI) is one the most prevalent bacterial infection and Escherichia coli has been isolated from the majority of UTI cases. On the other hand, the rate of community acquired UTI caused by the ESBL producer E. coli, is increasing worldwide. We aimed to assess the pattern of antibiotic resistance and the frequency of ESBL genes in the E. coli isolated from UTI of out-patients.

Methods: in this study, 140 E. coli were isolated from the urinary samples in 2012. The susceptibility of isolates to 10 selected antibiotics was tested using the disc diffusion method followed by the ESBL confirmation using the combined discs method. Finally, the ESBL genes were determined by PCR.

Results: out of 140 isolates, 81.43 and 62.13 percent showed resistance to ampicillin and Co-trimoxazole, respectively, but 100% were sensitive to imipenem. Moreover, 34 (24.28%) isolates were ESBL producers and 30(88%) and 18 (12.8%) isolates harbored blaCTX-M and blaTEM gene respectively. None of isolates contained blaSHV gene.

Conclusion: resistance to various beta-lactam antibiotics in particular the third generation of cephalosporins is a serious concern. The production of ESBL by community acquired strains is a big threat for the use of these antibiotics. Given the presence of ESBL genes in the high proportion of the isolates, more molecular and epidemiological studies on bacterial pathogen in this region is required.

Keywords: urinary tract infection, Escherichia coli, ESBL

P19: **Molecular Detection of blaIMP and blaVIM and blaSPM-1 Genes in Pseudomonas aeruginosa Isolated in Baghdad, Iraq**

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Background and Aim: Metallo-β-lactamase (MBL) producing Pseudomonas aeruginosa have been reported to be an important nosocomial infections. Its intrinsic and acquired resistance to many antimicrobial agents and its ability to develop multidrug resistance imposes a serious therapeutic problem. The aim of this study was to determine the molecular characterization of MBL- producing P. aeruginosa isolates obtained from different clinical samples in Baghdad province, Iraq.

Methods: Different clinical samples were collected from public and private hospitals in Baghdad city. Bacterial identification was done using conventional cultural, biochemical tests, and VITEk 2 system. Minimum inhibitory concentration (MIC) testing was performed using VITEK 2 automated system. Each P. aeruginosa isolates showed resistance to Carbapenems (Imipenem and Meropenem) were subjected to Imipenem-EDTA combined disc synergy test (CDST) to investigate the production of MBL (confirmative test). The presence of bla-genes encoded IMP, VIM, SPM-1 was detected by conventional PCR technique.

Results: A total of 75 P. aeruginosa isolates were isolated, 16 (21.3%) were able to grow on MacConkey agar supplemented with Meropenem 4mg/L (MMAC). The MIC of different antibiotics showed that 6 (37.5 %) isolates were Carbapenem resistant, MIC ≥16 µg/ml, while 4 (25%) isolates appear to be MBL producer using CDST test. PCR assay revealed that 3 (50%), 1 (16.6%) of the carbapenem resistant isolates harbored blaIMP, blaSPM-1 genes, respectively. blaVIM gene was not detected in this study. Discussion: The MBL producing P. aeruginosa isolates were more resistant to various antimicrobial agents. This suggests that MBL producing isolates in hospitals may cause serious infections that illustrated when these strains were responsible for a nosocomial outbreak.

Conclusion: The prevalence of multi-drug resistant P. aeruginosa isolates especially Carbapenem resistant bacteria was increased in Baghdad province. The blaIMP was the predominant among the MBLs genes in P. aeruginosa in this study.

Keywords: Pseudomonas aeruginosa , MBL, Carbapenems, ESBL, CRPA, bla genes

P20: **Study of Antibiotic resistance pattern in Klebsiella pneumoniae strains isolated from clinical samples by disk diffusion method, phenotypic detection of ESBLs and determination of minimum inhibitory c**

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Background and Aim: Introduction and Objectives: One of the mechanism of antibiotic resistance in gram negative bacteria, particularly Klebsiella pneumonia strains, is producing Extendet-Spectrum β lactamase enzymes (ESBLs). Encoding genes of ESBLs are usually located on the plasmid and they have ability to transfer to other gram-negative bacteria. Thus, due to the importance of resistance pattern recognition and it's sensitivity to the β- lactam antibiotics, the above issue was examined in this study.

Methods:: In this study different clinical samples of Boroujerd and Hamadan Hospitals during 6 month were collected and identified by biochemical tests and Enterosystem kit. Antibiotic resistance by Disk diffusion method was performed .Phenotypic Confirmatory Test for the presence of ESBLs was used. MIC antibiotics of Ceftazidime and imipenem by E test method was determined

Results: The results showed that the highest rate of Klebsiella pneumoniae strains resistance was related to Cefexime antibiotics (CFM) (46.7%), Ceftriaxone (CRO) (43.3%), Azthrunam (43.3%), Cefotaxime (41.7%), Cotrimaksazol (40.8%) , Ceftazidime (36.7%) and the least resistance was related to antibiotics Imipenem (0%) Sprofluksasin (16.7%), Cefepime (25%) and Gentamicin (26.7%).

Conclusion: The high prevalence of antibiotic resistance and ESBLs production in the studied cities indicating the need for screening of ESBLs in clinical samples by laboratory and the use of appropriate antibiotics with β-lactamase inhibitory power and antibiotic combination with Clavulanic by physicians.

Keywords: Klebsiella pneumoniae, antibiotic resistance, beta-lactamase, ESBLs

P21: **Antibacterial properties of extract root gentiana plant on three bacteria Escherichia coli, Streptococcus and Staphylococcus**

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Background and Aim: Gentiana lutea is herbaceous and tall plant which it roots has sinks in the soil deeply and its including componentssuch as gentiomarin, acid gentiozic and gentionozid.

Methods: The root of this plant has many pharmaceutical properties such as fever in malaria, facilitating the operation excrete and anemia decrease.

Results: . In this scheme, the root extract gentiana (after extraction in vacuum) were added to agar mediums containing Streptococcus, Escherichia coli and Staphylococcus. The aim of this work was to investigate the antibacterial activities of root gentiana

Conclusion: The results indicatethat this extract has positive effect on three bacteria.

Keywords: Rat, gentiana, root, bactery, extract

P22: **Analytical Sensitivity and Specificity of New Designed ureC Based PCR assay for Detecting of Helicobacter pylori in Biological and Environmental Samples**

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Background and Aim: Helicobacter pylori (H. pylori) is a etiologic agent of gastritis, peptic ulcer disease, and a risk factor for mucosa associated lymphoid tissue lymphoma and gastric adenocarcinoma. Rapid diagnosis and treatment of H. pylori presents a challenge. The application of polymerase chain reaction (PCR) with respect to H. pylori is useful for molecular epidemiologic aspects and detection purposes. The goal of the present study was to define highly conserved region of the ureC sequence that is share among most H. pylori strains and to develop and validate specific and sensitive PCR methods for the detection of H. pylori in various samples.

Methods: In silico and molecular approaches was used to achieve sensitive and specific detection of H. pylori. A multiple alignment of ureC (glmM) gene sequences deposited in the GenBank database was performed using ClustalW2 software. Specific primers were designed using conserved region of ureC gene obtained by alignment on the basis 26695 (Accession No. AE000511) as a template. Optimal PCR reaction was carried out according to conventional protocol. The PCR products were subjected to electrophoresis and stained with ethidium bromide. PCR detection limit (detectability) for newly designed primer was determined using tenfold serial diluted DNA of H. pylori with known concentration. For the purpose of calculating copy number of H. pylori detection limit, the average genome size of this DNA was estimated to be 1.67×106 base pair. In fact 1.67 fg of DNA is equivalent to a single genome of H. pylori. The specificity of the primers was tested by their ability to correctly amplify the gene of interest and then demonstrated by testing the PCR assay on at least 100 ng of genomic DNA isolated from battery of bacteria other than H. pylori which maybe present in biological and environmental samples. PCR amplification was considered negative if no amplicon was detected.

Results: The primers have 100% identity with most reference strains of H. pylori in BLAST searching. PCR analysis with specific designed primers amplified an approximately 214 base pair DNA fragment of ureC. The minimum concentration of pure extracted H. pylori DNA which yielded a detectable band on the agarose gel after PCR amplification using the primers was 10 fg per reaction. Since the average genome size of H. pylori DNA is estimated to be 1.67×106 base pair, 1.67 fg of DNA is equivalent to a single genome of H. pylori. Thus limit of detection in the PCR is equivalent to approximately 6 copy number of H. pylori per reaction. The positive reaction was obtained only with H. pylori DNA and there is no detectable band if non-H. pylori DNA were subjected to amplification.

Conclusion: In summary, the results of the study indicate that new designed ureC based PCR assay is a promising diagnostic molecular test, with excellent analytical accuracy and sensitivity. The test could easily be applied for the diagnosis of H. pylori in various samples.

Keywords: Helicobacter pylori, PCR, Analytical Sensitivity and Specificity

P23: **Molecular analysis of gene ERG11 from fluconazole- resistance of Candida albicans isolated suffering patients to vaginal candidiasis**

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Background and Aim: Drug resistance to azoles in especially fluconazole in candida abicans is due to different factors ,and molecular mechanisms that created these resistance one include excess expression of number of pumping genes towards out of cell or creating point mutation in ERG11 gene in target enzyme or resistance to excess expression. This study is evaluating nucleotide changes of ERG11 gene and is considered as one of purposed assumptions for gene resistance to Candida abicans against fluconazole.

Methods: 56 prepared samples from infected patients to vulvovaginit candidiasis have studied after culturing in medium of SDA and of chrom agar have grown proved that those yeast colony. Then based on NSSL protocol,MIC technical was preformed for determining sensitivity of identified yeast. The full duplicated genes ERG11 through Real-time PCR and identify fragments containing the SNP by the High Resolution Melting point difference was the presence SYT_9 color.The different groups based on the melting point of the sequence was determined by PCR-Sequencing.ERG11 gene sequences for each sample with normal sequence aligned with the software and identify the types of mutations in finally done

Results: We have seen in the survey conducted in 8 fluconazole-resistant yeast Candida albicans ERG11 gene mutations and changes in amino acid

Conclusion: Resistance to azole antifungal drugs in Candida species is now recognized as a major clinical problem. That may cause mutations in the ERG11 gene of Candida albicans resistant to fluconazole, ERG11 gene expression have seen Candida albicans of fluconazole resistance

Keywords: Candida albicans ,vaginal candidiasis ,ERG11 gene

P24: **Prevalence of qnr genes among clinical isolates of uropathogenic Escherichia coli in children**

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Background and Aim: Introduction: Urinary tract infection (UTI) is one of the most common childhood bacterial infections and E.coli is the major pathogen. The aim of this study was to determine the prevalence of qnr genes in E.coli strains isolated from UTIs

Methods: In this study, a total of 120 isolates of E.coli from urinary tract infections of the children, which were collected at BESAT hospital in Hamadan, west of Iran, from October 2010 to October 2011, included. The isolates diagnosis was done by standard biochemical methods. Antimicrobial susceptibilities were determined by disk diffusion method. The presence and identity of qnr genes were determined by polymerase chain reaction (PCR).

Results: The highest sensitivity was seen respectively to Ofloxacin (81.7%), Norfloxacin (70.8%) and Ciprofloxacin (79.2%); in contrast the highest rate of resistance was seen for Nalidixic Acid (40.9%).The results showed that 6(2.18%) and 4(1.12%) E.coli-producing isolates of ESBL were positive in case of existing qnrB and qnrS genes respectively. qnrA was not diagnosed in any isolates.

Conclusion: A high frequency of qnr genes among ESBL- producing E. coli was identified in this study.It is recommended that in order to avoid treatment failures, use phenotypic and molecular methods for experiments related to diagnose these enzymes and qnr genes.

Keywords: Escherichia coli (E.coli), Quinolones, UTI, Antibiotic resistance

P25: **Kinetics studies on the biosorption of Nickel and Zinc from industrial wastewater using klebsiella**

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2- Chemical Engineering Department, Arak University, Arak, Iran

Background and Aim: Introduction: metals, including inorganic contaminants in drinking water are considered dangerous due to the presence of high amounts of waste flows and their stability in the environment, their treatment is essential. Uptake by bacteria (Biosorption) is one of eliminating pollutants.

Methods: Materials and Methods: In this study, heavy metals nickel and copper from industrial wastewater treatment plants were investigated Arak Kheirabad area. In this study, the four points of the waste water treatment were sampled and bacterial analysis was performed. Bubble column bioreactor wastewater contains a concentration of 20 ppm of nickel and zinc were inoculated with isolated bacteria . To study the kinetics of the reaction, concentration data with pseudo-first-order and pseudo-second-order equation was evaluated.

Results: Results: klebsiella was isolated from samples and determined by microbiological methods. The bacterium could remove 96% zinc and 54% nickel, respectively in a bubble column bioreactor. Kinetic study showed that the standard deviation charts for biological uptake of nickel pseudo first and pseudo-second-order equation for 24/0 and 96/0. Similarly, the average rate on the pseudo first order and pseudo-second were to 47/0 and 99/0 respectively

Conclusion: Conclusion: The designed method could be biosorbed the studied metals not only in very short time as absorption reaction, but only absorbed in a continuous way due to supported continuous culture of bacteria in stress conditions. This method is purposed for routine work in industrial plants.

Keywords: heavy metals, nickel, zinc, biological uptake, kinetics, Bubble Tower

P26: **antibiotic sensitivity in leukaemic patients**

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3- Faculty of Paramedicine, Tehran University of Medical Sciences, Iran..

Background and Aim: Resistance to the antibiotics is the main cause of treatment failure in hematological malignancies. The aim of this study was to diagnose the bacterial strains isolated from blood specimens of cancer patients referred to Tabriz Shahid Ghazi hospital and determine their antibiotic susceptibility.

Methods: In this descriptive analytical study, totally 613 cancer patients especially leukemia patients were enrolled to the research. After obtaining 0.5 ml of venous blood from patients, blood cultures and determination of antibiotic susceptibility tests were performed using standard methods and BHI, EMB, Blood agar and Muller Hinton agar media. Then, the isolated bacteria were taken under the antibiogram tests using necessary antibiotics.

Results: Out of 613 cultured specimens, 153 cases were found to be positive including 76.47% of gram negative and 23.53% of gram positive bacteria. The most common isolated bacteria were E. coli, coagulase-negative Staphylococci, Klebsiella, Staphylococcus aureus and Pseudomonas aeroginosa respectively. The antibiogram tests demonstrated that E. coli susceptibility to gentamicin was the maximum whereas to the ampicillin was the minimum.

Conclusion: It seems that cephtriaxon is a good choice for bacteremia caused by gram negative bacteria whereas gentamicin can be a good drug for bacteremia caused by gram positive agents. It appears that due to the high resistances of bacteria to used antibiotics in this study, cases such as early starting use of antibiotics and also using of non-efficient doses of drugs must be considered to avoid increasing of bacterial drug resistances.

Keywords: Cancer, blood cultures, bacteria, antibiotic resistances

P27: **Inhibitory effect of tramadol on catalase of Pseudomonas aeruginosa**

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Background and Aim: Tramadol is an analgesic drug that binds to specific opioid receptors. It may contribute to the inhibition of neuronal re-uptake of noradrenaline. Catalase (CAT) (EC 1.11.1.6) is a key enzyme for degrading H2O2 in cells and it is a heme containing enzyme . In this research, the effect of tramadol on the activity of catalase of Pseudomonas aeruginosa was investigated . Lineweaver-Burk plot was applied to find out the inhibition pattern and kinetic parameters of the enzyme was calculated.

Methods: Pseudomonas aeruginosa was cultured in medium containing ethanol as carbon source. After 72 h (in the stationary phase), the cells were harvested and cell free extract was prepared. Tramadol was prepared with concentrations ranging from 0.65 to 3.8 mM as an inhibitor. For catalase activity assay ,Different concentrations of H2O2 as substrate (1 - 8 mM)was used for 10 min in the presence and absence of tramadol. The catalytic activity of CAT was measured at 240 nm using a UV-visible spectrophotometer.

Results: Tramadol could inhibit Pseudomonas aeruginosa catalase with mixed inhibition . The Km of enzyme was found about 6.6 mM and increased by increasing tramadol concentration and Vmax of enzyme was found about 0.37 mmol/min /mg protein . The Ki and IC50 values were determined as 0.45 and 1.5 mM for Pseudomonas aeruginosa catalase.

Conclusion: Catalase is a key enzyme for degrading H2O2 in cells. In this research, the effect of tramadol on the activity of catalase of Pseudomonas aeruginosa was investigated . Tramadol could inhibit Pseudomonas aeruginosa catalase with mixed inhibition . Lineweaver-Burk plot was drawed and kinetic parameters of the enzyme was calculated. The Km of enzyme was found about 6.6 mM and Vmax of enzyme was found about 0.37 mmol/min /mg protein . The Ki and IC50 values were determined as 0.45 and 1.5 mM for Pseudomonas aeruginosa catalase

Keywords: Drug, Enzyme, Inhibition, Bacteria.

P28: **Frequency of Toxic shock Syndrome Genes and Methicillin Resistancy in Clinical isolates of Staphylococcus aureus Isolated from Hospitalized Patients in Teaching Hospitals of Qazvin and Tehran.**

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Background and Aim: Staphylococcus aureus is one of the most common bacterial pathogens. TSST-1 and enterotoxins are the major virulence factors of this organism which are superantigens and cause toxic shock syndrome (TSS). The toxin-encoding genes located on pathogenicity islands with the ability to move to non-toxigenic strains to turn them as pathogenic bacteria. High Antibiotic resistance especially resistance to methicillin , causes problem in treatment of infection due to methicillin resistant S.aureus (MRSA). The aim of this study was to determine of frequency of most common genes (tst, entB and entC) causing toxic shock syndromes and resistance to methicillin in clinical specimens of Staphylococcus aureus isolated from hospitalized patients .

Methods: In total, 200 isolates of Staphylococcus aureus were collected from hospitalized patients, over a period of 11 months. All isolates were initially identified using standard biochemical testes and then were confirmed by detection of the femA gene which is intrinsic for Staphylococcus aureus. PCR assay was performed for detection of tst , entB and entC genes. Resistance to methicillin detected by agar screen test.

Results: Of 200 isolates, 41 isolates (20.5%) were positive for tst and entC genes. Among them, 31 isolates (15.5%) were positive for tst, 6 (3%) for entB and 4 (2%) for entC genes. 2 isolates (1%) were also positive for both tst and entC genes. Out of 200 isolates of S.aureus 120(60%) were resistant to methicillin and 10 (32.2%) of harboring tst genes isolates were MRSA

Conclusion: According of the results of this study showed the significant presence of toxic shock syndrome toxins genes and high antibiotic resistence in clinical isolates of Staphylococcus aureus in studied hospitals. Significant relationship between the isolates producing toxins and resistant to methicillin shows the clinical importance of these isolates and contributing them in public health.

Keywords: Staphylococcus aureus, tst, entB, entC , toxic shock syndrome , MRSA

P29: **Evaluation of three pair primers to detect Staphylococcal enterotoxin C genes**

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Background and Aim: More than 20 types of Staphylococcus aureus enterotoxin are introduced. Detection each of them is very important in clinical sample. Different detection primers have been designed and used. The aim of this study was to compare the efficacy of three types of primers to detect staphylococcal enterotoxin type C

Methods: In this study, three pair primers were selected. The first primer was amplified a 102 bp fragment. The second amplify a 700bp fragment and the last was amplified 1200 bp fragments. PCR product sequencing was carried out. Standard sequences were then compared and multiple alignment. Results were subjected to descriptive analysis.

Results: The results showed that each of the three primers and amplification may identify a target point. But anything other than toxin sequences may be amplified. The smaller amplified sequences increased probability of the non-specific amplification product. The Primers pair amplify a fragment of extra frame gene were more likely to encounter errors. While, inters gene fragments amplification pairs primer were shown more specific.

Conclusion: The results indicate that, in the case of different gene is present in many types, choosing specific primers to amplify the small fragments are not reliable. Whereas that the extra gene pair primers were designed to amplified a large fragment of within the gene is %100 specified.

Keywords: Staphylococcal enterotoxins, PCR, alignment, specificity

P30: **Molecular and serological assay on Staphylococcal enterotoxin C**

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3- Department of Rheumatology, Baqiyatallah University of Medical Sciences, Tehran, Iran.

Background and Aim: Staphylococcus aureus is Gram-positive bacteria that cause Broad range of infections in humans. In most cases, coagulase production has been considered as a virulence factor and coagulase- negative strains are discarded. While, coagulase- negative strains capable of producing enterotoxin (superantigen) which are Taken into consideration as pathogenic bacteria with virulence factors. So, provides a rapid and accurate diagnosis method is a priority of the health system. The aim of this study is molecular and serological detection of Staphylococcal enterotoxin C

Methods: In this study, a standardized Staphylococcus aureus producing enterotoxin C were subjected to gene extraction and the PCR detection was set up by use of amplified primers 102 bp fragment. Then, out of 50 samples of Staphylococcus aureus collected from different sources were investigated. Also, all strains were separately cultured in BHI- broth and production of enterotoxin C was examined by ELISA method. The data were descriptively analyzed

Results: The result of PCR test was ampilified the 102 bp fragment and gene sequencing was confirmed Enterotoxin type C gene. Study of 50 Clinical samples indicates that several of them have Enterotoxin C genes. The ELISA results showed that all strains containing enterotoxin C gene are able to produce this toxin

Conclusion: Based on the findings of this study, Staphylococcus aureus enterotoxin C have been detected in clinical samples by use of molecular PCR method and ELISA test and their involvement in the occurrence of disease is shown. In addition, Coagulase-negative strains isolated from clinical or food samples that are toxigenic should not be set aside

Keywords: Enterotoxin type C, PCR, ELISA, Staphylococcus aureus

P31: **Phenotypic and genotypic determination of erythromycin resistance transposons in Streptococcus pneumonia isolated from clinical specimens**

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2- Research Center for Applied Microbiology, Baghiatallah University of Medical Sciences, Tehran, Iran

Background and Aim: Streptococcus pneumonia is a globally significant pathogen and a significant increase in the rate of erythromycin resistance among Streptococcus pneumonia isolates has been reported worldwide in the past decade. The most common transposons harboring erythromycin resistance genes are Tn6002/ Tn3872, Tn6003 /Tn1545, Tn917. This study performed to determine erythromycin resistance relevant transposons.

Methods: A total of 41 erythromycin resistant Streptococcus pneumonia were collected from clinical samples. Disk diffusion method was performed for determining susceptibility to erythromycin, tetracycline and kanamycin. DNA were extracted by DNA extraction Kit were obtained from Peqlab company. Confirming the presence of transposon were performed by xis gene for excisas enzyme common in erythromycin related transposons.

Results: Antibiotic susceptibility tests were revealed that 89% and 91 % of erythromycin resistant isolates were resistant to tetracycline and kanamycin too respectively . These results showed the presence of 37 (82 %) isolates containing Tn6003 /Tn1545 and 3 (7%) isolates containing Tn6002/ Tn3872 transposons. PCR were showed the presence of xis gene in all isolates (%100).

Conclusion: These results showed the high prevalence of erythromycin resistance harboring transposons in clinical isolates in Iran. On the other hand simultaneous resistance to tetracycline and kanamycin were providing limited treatment choices.

Keywords: Streptococcus pneumonia ,erythromycin resistance ,transposons

P32: **Phenotypic and genotypic determination of erythromycin resistance transposons in Streptococcus pneumonia isolated from clinical specimens**

Azadeh Azadegan[1], Ali Ahmadi[1,2], Javad Sadeghi[1], Malihe Talebi[2]

1- Department of Medical Microbiology, Faculty of Medical Sciences, Tehran University of Medical Sciences, Tehran, Iran
2- Research Center for Applied Microbiology, Baghiatallah University of Medical Sciences, Tehran, Iran

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Conclusion: These results showed the high prevalence of erythromycin resistance harboring transposons in clinical isolates in Iran. On the other hand simultaneous resistance to tetracycline and kanamycin were providing limited treatment choices.

Keywords: Streptococcus pneumonia ,erythromycin resistance ,transposons

P33: **Prevalence of qnr Genes in Klebsiella pneumoniae in Khorramabad, Iran**

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2- Department of Microbiology, Qom Branch, Islamic Azad University , Qom, Iran.

Background and Aim: Klebsiella pneumoniae has been associated with different types of infections and one of the most important aspects of Klebsiella pneumoniae is the emergence of quinolone resistant strains. This study was undertaken to investigate the prevalence of quinolone resistance in Klebsiella pneumonia with different antibiotic susceptibilities.

Methods: Totally, 85 Klebsiella pneumoniae isolates were collected, from hospitalized patients in Khorramabad, from December 2012 to September 2012. Isolates were from different clinical samples including urine, sputum, pus, etc. Biochemical characterizations were performed for confirmation of isolates. Antibiotic susceptibility testing by disk diffusion method was performed according to Clinical and Laboratory Standards Institute recommendations using 12 antibiotic discs. K. pneumonia isolates were further tested for qnrA, qnrB and qnrS by multiplex PCR.

Results: Out of 85 isolates, 34 isolates were multi-drug resistant (MDR). The highest rate of resistance was observed in Ceftazidime and Cefotaxime. Moreover, the lowest rate of resistance was observed in Imipenem, Meropenem and Amikacin, respectively. Ciprofloxacin (Quinolone) susceptibility testing showed that 27 isolates were resistant, 6 isolates were intermediately resistant and 52 isolates were sensitive. Among the isolates, 14(17%) isolates were qnr-positive in which 11 isolates housed the qnrB, 1 isolate qnrA and 1 isolate qnrS. The other isolate possessed both qnrB and qnrS genes. Out of qnr-postive isolates 69.2% were among MDR strains and 27% of qnr-positive isolates were resistant to Ciprofloxacin.

Conclusion: Our study showed high frequency of qnr-positive K. pneumonia. Moreover, qnr genes which have detected in clinical isolates of K. pneumonia indicates that qnr genes are disseminating and the prevalence of MDR K. pneumonia is increasing because of probable increasing resistance to quinolone.

Keywords: Klebsiella pneumoniae, antibiotic susceptibilities, qnr, clinical

P34: **The first report of qacEΔ1 Gene in Klebsiella pneumoniae Clinical Isolates in Iran**

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3- Razi Herbal Medicines Reaserch Center, Lorestan University of Medical Sciences, Khorramabad, Iran.

Background and Aim: Biocides play a significant role in infection control by being used as disinfectants. Recently, the links between biocide resistance and antibiotic resistance have been recognized. The resistance to biocides causes microbial changes that result in antibiotic resistance. This study verified the presence of qacEΔ1, a determinant of resistance to quaternary ammonium compound (QAC) disinfectants, and evaluated the antibiotic susceptibility of Klebsiella pneumoniae clinical isolates.

Methods: Eighty five K. pneumoniae strains were isolated from different clinical samples including urine, sputum, pus, etc. Isolates were collected between December 2012 and September 2012 and confirmed by biochemical characterization. Antibiotic susceptibility testing by disk diffusion was performed according to Clinical and Laboratory Standards Institute recommendations using 12 antibiotics. Susceptibility of isolates to a biocide was also determined by micro plate dilution method. K. pneumoniae isolates were further screened for qacEΔ1 gene by using PCR amplification.

Results: Antibiotic susceptibility testing showed that 40% (34 isolates)of isolates were multi-drug resistant (MDR). Among 12 antibiotics tested, the highest level of resistance was to Ceftazidim, Cefotaxime, Cefixime, Cefteriaxon and Amoxiclave; Furthermore, the lowest level of resistance was to Imipenem, Meropenem, Amikacin and Tetracycline respectively. The results indicated that 25 of 85 isolated were qacE?1-positive of which 65.2% were among MDR isolates. Moreover, out of qacE?1-positive isolates, 69.5% were among biocide-resistant isolates.

Conclusion: The proper use of biocide is a cornerstone of any effective program of prevention and control of health care-associated infections. Our study showed high frequency of qacEΔ1 in MDR K. pneumoniae, therefore improper usage of biocide can probably lead to biocide resistance and higher antibiotic resistance in K. pneumoniae. So, this issue is of special concern. This is the first description of qacEΔ1 in K. pneumoniae in Iran.

Keywords: qacEΔ1, K. pneumoniae, Biocide, Antibiotic Resistance

P35: **Drug Resistant and the role of Microbiology Laboratories**

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3- MD.Microbiology Department,Tehran University,IRAN
4- 7-Assistant Professor,Quality Management and Accreditation Office,Reference Health Laboratory,ministry of Health and Education,IRAN.

Background and Aim: The aim of this study was to determine drug –resistant pattern and prevalence of antimicrobial resistant organisms in one-year duration in admitted patients. BACKGROUND: The increase prevalence antimicrobial-resistant organisms is major health problem and is particular concern of hospitals and other care settings.there are a few search for antimicrobial resistant but there is not Drug-Resistant pattern ,up to now.Antibiotic using monitors in the other country to prevent drug resistant and it is necessary for us.

Methods:: We evaluated admitted patients in hospital during May 2011 to May 2012.Samples included urine-culture and excluded Urine-culture.We cutured and then selected antibiotic disks for disk-diffusion methods.

Results: The most frequent isolated pathogens were Eschercia coli (32%)followed by Staff aureus (26%)and then Klebsiella (11%).High rate of resistance is for E.coli that prediction of ESBL was found 42% and for all Gram-negative Bacilli was 38%.

Conclusion:: Antimicrobial resistant pattern observe resistance trends that would influence appropriate empire treatment and infection control strategies for bacteriamic disease. Identifying the resistance pattern of microorganisms in every hospital is the key to success in the appropriate patients treatment.

Keywords: Antibiotic,Antimicrobial Resistant,Drug-Resistant

P36: **Microbiology Laboratory Quality Control in Tehran Hospitals**

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Background and Aim: This study attempts to clarify the concept of Laboratory Quality Management System (lab QMS) for a medical testing and diagnostic Microbiology Laboratory in a holistic way and hopes to expand to horizon beyond Quality Control (QC) and Quality Assurance. We tried to show the Quality Control in Microbiology Laboratories in capital hospitals. QMS in Microbiology Laboratories was instructed in the other country but it is not completed in IRAN and it seems QMS is a new subject.

Methods: we prepared map plan for all hospitals in capital.The questionnair approved by Ministry of health and medical education.we were done grouping the hospitals and date of implementation .it followed by filling the questionnair,speaking with boss,auditing the organization based on ISO 15189 ,QMS and analyzing data .

Results: The survey was seen the rate of Quality Control in hospitals has incompletely form,all the organizations followed the international standards for Microbiology ,checking the stain,using different strains during the procedure,but it has a poor monitoring the stages and calibration of instruments.

Conclusion: A common problem with most of laboratories is that within a day of receiving the status edited laboratory their Quality practices slump back to primitive levels till a few months from neat assessment when they again wake and make a dash for Quality. Likely case is the change in exciting staff with most worrisome being change in the Quality Management (QM).

Keywords: Quality Control,Quality Management System,Quality Assurance,Microbiology Laboratories,ISO 9001,ISO 15189

P37: **Antimicrobial Activity of Rheum ribes Extracts against Some Gram Positive and Negative Bacterial Strains**

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Background and Aim: Antimicrobial Activity of Rheum ribes Extracts against Some Gram Positive and Negative Bacterial Strains Morteza Azizollahi Aliabadi1, Reza Kazemi Darsanaki2 1Department of Microbiology, Faculty of Basic Science, Lahijan Branch, Islamic Azad University, Lahijan, Iran 2Young Researchers Club, Lahijan Branch, Islamic Azad University, Lahijan, Iran Abstract The use of plant compounds to treat infection is an age-old practice in a large part of the world, especially in developing countries, where there is dependence on traditional medicine for a variety of diseases. According to World Health Organization medicinal plants would be the best source to obtain a variety of drugs. Rheum ribes is among the Polygonaceae family which is endemic in Iran and a few neighboring countries. In this investigation, antibacterial effects of root, leaves and stalk methanol and aqueous extracts of Rheum ribes against some gram positive (Staphylococcus aureus, Bacillus subtilis) and negative bacterial strains (Escherichia coli, Klebsiella pneumoniae, Pseudomonas auregnosa, Shigella flexneri) were studied, using well diffusion method. Methanol and aqueous extracts obtained from root, leaves and stalk of Rheum ribes exhibited antimicrobial activity against test microorganisms at various rate. Rheum ribes Extracts were found to be most active against Shigella flexneri and Klebsiella pneumoniae with inhibitory, 11.5 and 10.70 mm. The results suggested that extracts of Rheum ribes could be effectively used against diseases caused by selected human pathogens. Keywords: Antibacterial activity, Zone of Inhibition, Rheum ribes

Methods: In this investigation, antibacterial effects of root, leaves and stalk methanol and aqueous extracts of Rheum ribes against some gram positive (Staphylococcus aureus, Bacillus subtilis) and negative bacterial strains (Escherichia coli, Klebsiella pneumoniae, Pseudomonas auregnosa, Shigella flexneri) were studied, using well diffusion method. Methanol and aqueous extracts obtained from root, leaves and stalk of Rheum ribes exhibited antimicrobial activity against test microorganisms at various rate.

Results: Rheum ribes Extracts were found to be most active against Shigella flexneri and Klebsiella pneumoniae with inhibitory, 11.5 and 10.70 mm.

Conclusion: The results suggested that extracts of Rheum ribes could be effectively used against diseases caused by selected human pathogens.

Keywords: Antibacterial activity, Zone of Inhibition, Rheum ribes

P38: **Efficiency of Naloxone/Alum mixture as adjuvant on methicillin-resistant Staphylococcus aureus vaccine**

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Background and Aim: The prevalent epidemic of hospital and community-acquired methicillin-resistant staphylococcus aureus (MRSA) infection has caused principal human morbidity, such as osteomyelitis, endocarditis and pneumonia. Because of drug resistant and ineffectiveness of vaccines, there is a requirement for improved vaccine development. Previous studies indicated that Alum could induce humoral immunity, but it couldn't cellular immunity. Here, mixture of naloxone and alum was used as a novel immunomodulator for increasing vaccine efficacy.

Methods: Six-to-eight weeks old female BALB/C mice were divided into 7 groups. Vaccine was prepared via sonication of methicillin-resistant staphylococcus aureus (MRSA) bacteria. First group of mice received vaccine with naloxone. Second group of mice received vaccine and naloxone/ alum mixture. Mice in the third group received vaccine/alum. Mice in the fourth group immunized with vaccine in Freund's adjuvant. Fifth group of mice received vaccine alone; Mice in the six group immunized with naloxone alone and in the seventh group (negative control group) received phosphate-buffer Saline. Booster immunizations were carried out at weeks 2 and 4. Two weeks after the last injection, immune responses were evaluated. Lymphocyte proliferation analyses were used via Brdu method. IL-4 and IF-ɣ cytokines, total antibodies and IgG1, IgG2a isotypes were evaluated with ELISA method.

Results: Results demonstrated that naloxone/alum mixture as an adjuvant enhanced the ability of the MRSA vaccine to increased lymphocyte proliferation and Th1 immune responses. Also were enhanced total antibodies and IgG1, IgG2a isotypes.

Conclusion: In conclusion, use of MRSA vaccine formulated in naloxone/alum mixture as adjuvant, could increase both cellular and humoral immunity and conduct the immune responses toward Th1 pattern.

Keywords: Methicillin-resistant staphylococcus aureus, naloxone/alum mixture, Vaccine.

P39: **Study of bacteria causing skin infections and antibiotic resistance in patients referred to Shohada and Loghman hospitals in 1389-90 years.**

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Background and Aim: Diagnosis of bacterial causes of cutaneous infections and determination of their resistanc to different antibiotics is required for choosing the optimal treatment. This study was performed on bacterial cultures which were taken from patients with cutaneous infection.

Methods: Sampeles were collected from 100 patients with cutaneous infection referred to clinical laboratories in Shohada and Loghman in 1389-1390. The samples were cultured by routine microbiological methods for isolation of microbial agents. The antibiogram disc agar diffusion technique was used to evaluate the sensitivity of microbial agents to antibiotics.

Results: The bacterial causing cutaneous infection in the order of their prevalence included Staphylococcus aureus (31%), Escherichia coli (22%), coagulase-negative Staphylococci (12%), Streptococcus spp (8%), Enterobacter (8%), Klebsiella (5%), Acinetobacter (5%), Pseudomonas spp (4%), Enterococcus (3%) and Proteus (2%). The most resistance organisems were Acinetobacter (86%), Enterobacter (81.7%), Enterococcus (78.4%), and the most sensitive organisems were Proteus (61.1%) and Streptococcus spp (60.7%).

Conclusion: Staphylococcus aureus is the most common microbial agents of cutaneous infection. In order to prevent bacterial resistance to antibiotics we must prescribe antibiotics based on bacterial culture and antibiograms.

Keywords: Cutaneous infection; Bacterial resistance; Staphylococcus aureus.

P40: **In silico selection of the best immunogenic surface iron repressed protein from Acinetobacter baumannii as a vaccine candidate**

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Background and Aim: Acinetobacter baumannii is a gram-negative bacterium that causes serious infections in compromised patients. Iron is an essential nutrient playing a role in bacterial differential gene expression and protein production and iron repressed proteins represent outer membrane siderophore receptors. This micronutrient plays an essential role in a diverse number of cellular processes including electron transport, nucleic acid biosynthesis, and protection from free radicals. Low free-Fe concentration stimulates active iron-acquisition systems and expression of genes coding for virulence factors such as hemolysins, toxins, and proteases. This condition is provided in cells cultured in the presence of DIP. In Gram-negative bacteria, iron-regulated gene expression is generally under the control of the Fur. A. baumannii grown under iron limited conditions resulted in major transcriptional changes of not only many iron acquisition related genes, but also genes involved in other processes such as motility. Overall, the above study showed that A. Baumannii is well adaptable to growth in an environment which has limiting iron availability. One strategy that has been explored to bypass the bacterial adaptation to drugs is to target the iron metabolism of bacteria, since iron is critical for all bacteria to grow.

Methods: Eight iron repressed Surface proteins were found by Chika Nwugo and colleagues in a proteomics research, The acinetobactin BauA receptor, enterobactin receptor protein FepA, ferric aerobactin receptor, a FhuE-like ferric rhodotorulic acid receptor, a BtuB-like vitamin B12 receptor. A protein annotated as an outer membrane siderophore receptor, outer membrane protein CarO which plays a role in antibiotic resistance and ornithine transport , the AtpA F0F1 ATP synthetase α subunit, a cytoplasmic membrane protein that was enriched with the OM fraction. Antigenicity of these proteins were analyzed by Antigen pro and vaxijen software. IEDB software also predicts immunogenic properties such as B cell epitopes, the hydrophilicity, the antigenicity, the flexibility, availability and Beta turns in proteins. Proteins with top scores from these software were selected for next step. homology of proteins with other bacterial strains and cross-reactivity was determined using protein blast with non redundant protein database. Solubility of the proteins were then predicted using SOLpro, the Recombinant Protein Solubility and PROSO software and finally allergenicity of selected protein was checked by APPEL software.

Results: The FepA protein because of high immunogenic scores, non allergenicity, solubility and high cross-reactivity is introduced as a suitable vaccine candidate against Acinetobacterbaumannii.

Conclusion: FepA can be use as a immunogen and protective vaccine candidate against Acinetobacter baumannii. In vitro immunization of this protein can be tested.

Keywords: Acinetobacter baumannii, iron, immunogenicity, FepA

P41: **Three dimentional structure prediction of Acinetobacter baumannii OmpA**

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Background and Aim: Acinetobacter baumannii is a rapidly emerging nosocomial pathogen causing infections with high mortality rates due to inadequate available treatment and remarkable capacity to acquire antimicrobial resistance attributable to its biofilm formation ability. The outer membrane protein A of A. baumannii (AbOmpA) is the most abundant surface protein associated with the apoptosis of epithelial cells through mitochondrial targeting. The N-terminal region of AbOmpA 22-170 was reported to be responsible for host cell death. OmpA plays important roles in anchoring of the outer membrane to the bacterial cell wall. OmpA was identified as the primary target of humoral immune response after intravenous infection by A. baumannii in mice. This protein has two-domains consisting of a predominantly β-stranded N-terminal domain with a globular C-terminal domain containing a high proportion α-helices. The C-terminal periplasmic domain of OmpA (OmpA-like domain) associates with the peptidoglycan (PGN) layer noncovalently. N-terminal β-barrel domain composed of eight membrane-spanning antiparallel, amphipathicb-strands connected by four long loops at the outer surface of the membrane and three short turns at the periplasmic face. The structure consists of an eight-stranded β-barrel connected by tight turns on the periplasmic side and larger mobile loops on the extracellular side. Vaccination of mice has been reported to markedly improve survival and reduce bacterial burden in mice infected intravenously. The anti-OmpA antibodies levels correlated with survival in mice. Passive transfer with immune sera recapitulated protection. 3D structure determination studies on A.baumannii OmpA have not yet been carried out.

Methods: In the present in silico study the protein sequence was extracted from NCBI database. Domain prediction was performed. A template for structure prediction of protein was selected with protein sequence blast against PDB database, a database for proteins with known structures typically obtained by X-ray crystallography or NMR spectroscopy. Protein template was selected focusing on its coverage length and identity percentage with the sequence under this study. 3D structures of periplasmic and transmembrane domains were determined with various software. All three-dimensional structures were evaluated by Qmean, a composite scoring function which is able to derive both global (i.e. for the entire structure) and local (i.e. per residue) error estimates on the basis of one single model. The top three structures were selected and refined with kobamin software that reduces the structures errors. The refined structures were evaluated with Qmean score and the quality of structures were observed to have improved.

Results: The best refined structure with the highest score was selected as the final structure. This structure was validated with other relevant software.

Conclusion: 3D structure ofAcinetobacter baumannii OmpA protein is predicted via in silico tools.

Keywords: 3D structure, Acinetobacter baumannii, OmpA

P42: **Application of penicillinase in validation of sterility test for four beta-lactam antibiotics**

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Background and Aim: Sterility is an essential test for injectable products in pharmaceutical industries. In this test, the inhibition effect of antibiotics should be neutralized. Beta-lactamase is a family of enzymes that breaks Beta-lactam ring. Penicillinase is one of type of these enzymes.

Methods: According to U.S. Pharmacopeia 34, in membrane filtration method for the sterility test of Beta-lactams, sufficient beta-lactamase should be added to the solvent, washing fluid and media. In the present study, sterility test of four beta-lactam antibiotics including: Cefazolin sodium, Ceftriaxone sodium, Ceftazidime sodium carbonate and Imipenem-cilastatin were conducted. Fluid A(1 gram/liter of peptone from meat)which had different dilutions of penicillinase and 10-100 CFU/ml of each microorganisms including S. aureus, P. aeruginosa, B. subtilis, Cl. sporogenes, C. albicans and A.niger, was filled in segregated bottles and then 20 vials of above mentioned antibiotics were solved separately. A sample of each bottle was counted by pour plate method. Then, the content of bottles were filtered by membrane filters with pore size 0.22µm. Filters were washed with fluid A without microorganisms which had the same dilution of penicillinase, and were put in Fluid Thioglycolate Medium(FTM) and Tryptone Soya Broth(TSB) with the same dilution of enzyme. FTM and TSB were incubated at 30-35 0C and 20-25 0C respectively. There were positive and negative controls for media and enzyme. Samples were incubated 3 days for the bacteria and 5 days for mould and yeasts. Each test was done three times.

Results: None of antibiotics had effect on C. albicans and A. niger. Regarding, P. aeruginosa growth observed in mentioned media containing all dilutions of enzymes. In presence of Cefazolin, Cl.sporogenes grew up when 14286 Unit/ml of enzyme was added; Meanwhile, growth of Cl.sporogenes was observed in presence of other above antibiotics without enzyme. In presence of Ceftriaxone and Ceftazidime, S.aureus was grown. In addition, S.aureus growth was observed in presence of Cefazolin and Imipenem-cilastatin, just after adding the enzyme more than 28571 unit/ml. Growth of B.subtilis observed in 14286 Unit/ml of enzyme in presence of Ceftriaxone and Ceftazidime and Cefazolin, but for growth of B.subtilis in presence of Imipenem-cilastatin, 85714 Unit/ml of enzyme needed to be added.

Conclusion: Although penicillinase could help to cleavage beta-lactam ring of mentioned antibiotics, our present study showed that: sterility test of these beta-lactams needs filtration, because no growth of bacteria in pour plate method was observed.

Keywords: Beta-lactamase,Sterility test,Beta-lactam antibiotics

P43: **The protective efficacy of Recombinant type B Flagellin of Pseudomonas aeruginosa in the murine burn wound model of infection**

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Background and Aim: Pseudomonas aeruginosa is typically considered to be an opportunistic bacterial pathogen. Infections with this organism are a major health problem for immune-compromised individuals such as burn patients. The bacterium has a single polar flagellum that can be classified into two serotypes, types A and B. These serotypes are distinguished based on reactivity with type-specific antisera. The flagellum is essential for bacterial motility, chemotaxis, invasiveness and adhesion. It can also activate the host inflammatory responses via Toll-like receptor 5 (TLR5). In this study, recombinant type B flagellin protein was produced and considered as a vaccine candidate.

Methods: Recombinant type B flagellin protein was produced and purified via Ni++ affinity chromatography. A total of sixty 6-8 weeks old male BALB/c mice were divided into five groups (n=12). The first two groups were immunized with 10µg of alum adjuvanted recombinant flagellin protein. The third group of mice received only the recombinant flagellin protein in PBS. The fourth and fifth groups of mice received alum and PBS as control groups. Experimental groups were boosted on days 14 and 28. Following the last injection, Lymphocyte proliferation was measured with Brdu method. IL-4, IFN-γ cytokines, as well as the level of total antibodies and their isotypes were evaluated using ELISA method. Also, experimental mice were burned according to the ethanol bath burn model described by Holder. The burned sites were then infected with lethal doses of P. aeruginosa culture. The mice were continuously monitored for signs of morbidity and mortality.

Results: Results showed that immunization with the recombinant flagellin B protein in mice increased the level of humoral and cellular immune responses and led to high protection against P. aeruginosa burn infection.

Conclusion: Recombinant type B flagellin is a good candidate as a vaccine against Pseudomonas aeruginosa infection.

Keywords: Pseudomonas aeruginosa, Recombinant type B flagellin, Vaccine Candidate, murine burn model.

P44: **Expression, Purification and Characterization of Recombinant type B Flagellin as a New Vaccine Candidate against Pseudomonas aeruginosa Infections**

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Background and Aim: Pseudomonas aeruginosa as an opportunistic pathogen that causes lethal infections in immunocompromised patients such as burn patients. This opportunistic pathogen has a single polar flagellum that has important roles in motility, chemotaxis, and establishment of P.aeruginosa in acute phase of infections. Flagellin is the major protein component of the flagellar filament that can be classified into two distinct serotypes; type A and B. In this study, we aimed to produce whole type B flagellin in bacterial E. coli host expression system.

Methods: The whole fliC gene sequence of P.aeruginosa strain PAO1 strain (flagellin encoded) was obtained from GeneBank. pET28a/fliC construct was produced by Biomatic; (Canada). it was transfered into the expression host E. coli BL21 (DE3). Following a four-hour induction of vector by 1mM IPTG, bacterial cells were collected. Purification of flagellin was performed via hybrid procedure of denaturing/Renaturing condition from inclusion bodies by Nickel-affinity chromatography. Purified protein was confirmed by SDS-PAGE and Western blot analysis using flagellin-specific antibodies were produced in rabbits.

Results: Recombinant type B flagellin was successfully expressed in E. coli BL21 (DE3) host. The protein electrophoresis showed that the molecular weight of recombinant type B flagellin is about 55 kD which is consistent with the bioinformatically predicted one. The Western blot analysis also confirmed the production of specificity of anti-type B flagellin. The amount of produced protein was measured by the direct spectrophotometery method (corrent protocols in protein sciences) which was 4mg/ml.

Conclusion: Since, the flagellin plays an important role in the primary establishment of P.aeruginosa in the burn infection; it seems that it can be used as a vaccine candidate in the sepsis model of infection.

Keywords: Pseudomonas aeruginosa, Recombinant flagellin, fliC , vaccine candidate.

P45: **Isolation and screening of cultivable marine bacterial symbionts within some sponges, collected from the Persian Gulf, for the anti-MRSA activity**

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Background and Aim: Marine sponges harbour diverse and abundant microbial communities so that these microbial associates can comprise as much as 40% of sponge tissue volume. Due to the strong selective pressure resulted from competition between marine bacteria associated with sponges for space and nutrient and the existence of a symbiotic relationship with their host, these bacteria were expected to be potential resources of novel bioactive products. The worldwide emergence and prevalence of methicillin resistant Staphylococcus aureus (MRSA) has become a serious clinical problem in 21st century, resulting in significant morbidity, mortality, and health care costs. So, the search for novel anti-MRSA agent is urgently needed. The purpose of this study was to screen of cultivable marine bacterial symbionts within some sponges, collected from the Persian Gulf, in order to finding the anti-MRSA compounds.

Methods: The sponge samples used in this study were collected from the Larak Island (Persian Gulf, Iran). In order to isolation of marine bacterial symbionts from sponge samples, the surface of sponges was sterilized with 80% ethanol and subsequently was washed three times with sterile seawater. Then under aseptic condition, the inner tissue of sponges was cut into approximately 1 cm3 cubes and subsequently the cubes were triturated and suspended with sterile seawater. Marine Agar 2216 was used for isolation of bacteria by serial dilution and pour plate techniques. All of the bacterial isolates were inoculated in Marine Broth and incubated on a rotatory shaker at 28 ˚C for 2-7 days. Anti-MRSA activity of their ethyl acetate extract was assessed at 100 mg/ml concentration using disc diffusion method against a MRSA strain collected from the Golestan hospital (Ahvaz, Iran). Synthetic antibiotic discs were used as control.

Results: A total of 21 bacterial isolates were obtained from sponge samples but among them only one light brown pigmented bacterium, identified as Pseudomonas sp. PG-03, was exhibited the capability of producing anti-MRSA compound. The PG-03 extract showed a relatively strong anti-MRSA activity. The diameter of the inhibition zones for vancomycin and PG-03 against MRSA were 18 and 29 mm, respectively. The optimized temperature and incubation time for anti-MRSA metabolite production by this strain were 22 ˚C and 96 h, respectively.

Conclusion: In conclusion, this study demonstrated that the antibiotic compound produced by Pseudomonas sp. PG-03 can gives hope in fight against MRSA and also, sponge-associated microorganisms can represent a potential resource for new drug development.

Keywords: MRSA, Antibiotic, sponge-associated microorganism, Persian Gulf

P46: **Ventilator-Associated Pneumonia and Antibiotic Resistance Patterns of Isolated Bacteria**

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Background and Aim: Ventilator–associated pneumonia (VAP) is the most important cause of nosocomial infection in mechanically ventilated patients. The objective of this study was to determine the incidence of Ventilator-associated pneumonia and to detect responsible bacteria & the pattern of their antibiotic resistance.

Methods: During November 2011 to September 2012, this cross-sectional study was performed on 100 patients (61 male & 39 female) who were admitted in the ICU ward of Khalij Fars Hospital, Bandarabbas, and required mechanical ventilation for at least 48 hours. Diagnosis of VAP was based on chest radiographic examination, clinical signs, arterial blood gases and microbiologic analysis of endotracheal secretion. All of the isolated bacteria were identified by biochemical tests and their antibiotic resistance were evaluated by disk diffusion method. SPSS 19 Software was used for statistical analysis.

Results: VAP was diagnosed in 15 patients (15%). According to findings the most common microorganisms responsible for VAP were Klebsiella pneumonia (27.8%), Pseudomonas aeroginosa (16.7%) and E.coli (16.7%). Isolation of one organism was seen in 80% and two organisms in 20% of subjects. Most of the isolated bacteria were resistance to ampicillin (92.8%), cefixim (92.3%) & co-Amoxiclav (83.4%) and the lowest resistance was to imipenem (33%), gentamycin (38%) and ciprofloxacin (40%). Mortality rate was 33.3%.

Conclusion: Our findings showed that the rate of VAP was relatively high and Klebsiella pneumonia was the most important cause of it. We also found imipenem, gentamycin & ciprofloxacin appears to be more effective antibiotics to treatment of VAP

Keywords: Ventilator–associated pneumonia, Nosocomial infection, Antibiotic resistance, Bacteria

P47: **Evaluation of antibacterial effects of essential oil of Achillea millefolium against Staphylococcus aureus and Enterococcus faecalis**

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Background and Aim: Some plant extracts, including species of millefolium have antibacterial effects andthey can be used as antimicrobial agents in treatment of infections. Hence, the aim of this study wastoevaluate the effect of essential oil and the anti-microbial properties of its essential oil.

Methods: In this experimental study, yarrow plant in late spring was collectedfrom Tabriz region in 2012. The compounds of the essential oil were analyzed by GC/MS. In this study,the minimum inhibitory concentration (MIC) and diameter of inhibition zone of growth for the standard strains of Staphylococcus aureus, and Enterococcus faecalis were determined throughdisk diffusion method and dilution in the liquid medium, respectively.

Results: Camphor was the major compound of the essential oil. The standard strains of Staphylococcus aureus presented the greatest sensitivity to the essential oil in MIC>2.365. In addition, it presented an intermediate sensitivity to standard strains Enterococcus faecaliswith MIC?4.73 to theessential oil. Also, the results of disc diffusion indicated that essential Achillea oil in %5 dilution has inhibitory effect of bacteria.

Conclusion: The essentialoil, especially possess anti-bacterial effects. According to the obtained results it is concluded that essential Achillea oil can be used as a factor for destroying the examined bacteria.

Keywords: Achilleamillefolium, Staphylococcus aureus, Enterococcus faecalis, Essential Oil.

P48: **Comparison of the effects of Au nanoparticles on pathogenic bacteria(MDR) prokaryote model and Wistar rats as a eukaryote model**

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Background and Aim: Nanoscale particles of gold now command a great deal of attention for biomedical applications. Despite the great excitement about the potential uses of gold nanoparticles for medical diagnostics, as tracers, and for other biological applications, researchers are increasingly aware that potential nanoparticle toxicity must be investigated before any in vivo applications of gold nanoparticles can move forward. In this paper the in vitro and in vivo toxicity of nano Au particles on bactrria and rat was investigated.

Methods: A total of 200 clinical samples in 4 hospital of Isfahan city was studied during 6 months. The MDR assay was performed by disk diffusion method. Minimum inhibitory concentration (MIC) values .A total of 32 healthy male Wistar rats of weighing (225± 25 g) were used. Animals were randomly divided into groups, three GNP-treated rat groups and one control group (CG). Group 1, 2 and 3 received. /5 cc of solution containing 5, 10,100 ppm nanogold via IP injection for 7 successive days, respectively. The control group was treated with 0.5% HPMC with same procedure. Then, several biochemical parameters such as serum glutamate oxaloacetat transaminase (SGOT) and serum glutamate pyrvate transaminase (SGPT) were evaluated at various time points (2, 7 and 14 days). After 14 days, the tissue of liver was collected and investigated

Results: Of the 200 patients studied, 144 (72.0%) had gram-negative bacilli containing ESBL and 56 (28.0%) had gram-negative bacilli without ESBL ,but only MDR and the most prevalent bacteria was identified as Klebsiella pneumonia, with especially strong resistance to cefotaxime. All of these bacteria were sensitive to the Au nanoparticle solution with density of 100 ppm, but the 10 nm size .In this study indicated that after 2 days, mean level of SGOT in group 3(Au100nm) significantly increased in comparison control group (p.v=0.028). Fourteen days after the injection of Au100ppm the liver damage returned. No significant effects were noted for SGPT after consumption of three doses of nanogold.Histopathological examination of liver in Group 1: degeneration of hepatocytes, aggregation of nucleus, basophilic in peripheral central vein- In group 2: atrophy of central vein, indiscrimination of lobule hepatic- shrink and hyperemia of central vein, hypertrophy of hepatocytes and remak line- In group 3: active hyperemia between lobules and in central vein, indiscrimination of lobule hepatic indicated.

Conclusion: The results seem to indicate a direct correlation between Au nanoparticle solution concentration and the diameter of growth zone for pathogenic bacteria(MDR). Assays in our study were in vitro; if use of Au nanoparticles in vivo proves to be with adverse effects, it could be a valuable alternative to antibiotics

Keywords: Gram-negative bacilli , MDR, Au nanoparticles, Wistar rat, Toxicity

P49: **Detection of A2142G and A2143G Mutations in 23SrRNA Gene of Helicobacter pylori Strains in Rasht**

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Background and Aim: Helicobacter pylori is a gram negative and microaerophilic bacillus that more than half the world's population has infected with this microorganism. H. pylori is causative agent of gastritis, duodenal ulcer, gastric ulcer, non-ulcer dyspepsia, MALT lymphoma and is associated with gastric adenocarcinoma. Clarithromycin is one of the antibiotics that use to eradicate the H. pylori. Point mutation in 23SrRNA gene on nt 2143 and 2144 when A turns to G is the most common mechanism which confer resistance to clarithromycin and leads to treatment failure. The aim of this study was to detect the A2143G point mutation in 23SrRNA of H. pylori strains isolated from gastric biopsies of patients in Rasht by PCR-RFLP.

Methods: A descriptive study was conducted on 89 DNA samples extracted from H. pylori strains isolated from gastric biopsies of patients who were suffering from one of the gastric disorders above mentioned. First of all, we confirmed all samples as H. pylori by amplifying ureC (glmM) gene. Then, by using specific primers we amplified 23SrRNA gene of all extracted DNA to perform PCR-RFLP using MboII and BsaI restriction endonucleases on the PCR product of 23SrRNA gene to find the frequency of point mutations on A2142G and A2143G.

Results: Results of PCR-RFLP showed that among 89 extracted DNA which were treated with BsaI and MboII restriction endonucleases, the point mutation on nt A2143G with the frequency of 5.6% was detected only by using of BsaI. These mutant strains were resistant to clarithromycin using Kirby-Bauer method. There was no point mutation in sensitive strains of Helicobacter pylori. Also, there was no point mutation on nt A2142G using MboII. Resistant frequency in samples isolated from gastric ulcer was higher than other gastric disorders. Women & patients with more than 60 years old showed the most resistance rate.

Conclusion: Frequency rate of clarithromycin resistance was lower than the other conducted studies in Iran. All resistant strains had A2143G genotype. It seems more studies need to determine other point mutations in other regions of 23SrRNA gene using other restriction endonucleases.

Keywords: Helicobacter pylori, clarithromycin resistance, PCR-RFLP.

P50: **Isolation and characterization of Burkholderia cepacia strains from hospitalized patients in western hospitals of Gilan province.**

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Background and Aim: Abstract Complexes of Burkholderia cepacia (BCC), formerly called the Pseudomonas cepacia is a plant pathogen that causes root rot, first in 1950 as an onion was described, already as a natural pesticide against disease other plants can be used it in a wet environment, soil and plants can be found. The bacteria rarely causes infection in healthy individuals and that can be from person to person and the body liquids, contaminated drugs or medical devices in hospitals found to be bacteremic BCC rarely reported is, this bacterium as an important factor and mortality in critically ill patients and patients with compromised immune systems due to the inherent resistance to antimicrobial known it. BCC isolates to most antibiotics that are normally used the antimicrobial resistance and the use of fast, effective treatment can reduce the mortality patients. The ability of B. cepacia than other bacteria to survive in the harsh environment of such that is a disinfectant. Positively identified species of this complex is important because some varieties of genomic (a particular genomic variants IIIA) are associated with high infection among patients and identify weak. Respiratory tract colonization by B. cepacia in patients with cystic fibrosis (CF) has been recognized since 1979 and is associated with significant morbidity and mortality of critically ill and over a third of the patients died within 6 months be and the remaining third in a significant drop in lung function be cause. During the fall quarter of 1391, a hypothetical 1 isolates from 90 patients with respiratory disease, bloodstream infections in the ICU and CCU patients and the monitoring device connected to the ventilator and had more than 3 days in the hospital West Gillan admitted using the optional BCSA (Selective Agar Burkholderia cepacia) was obtained, which was identified at the species level by biochemical tests, was isolated from a female patient with asthma.

Methods: We examined the capacity of Burkholderia cepacia selective agar (BCSA) as a medium for primary isolation of Burkholderia cepacia samples. Biochemical tests were used to confirm the identification.

Results: Burkholderia cepacia strains were isolated from 1 out of 90 samples as confirmed with biochemical tests.

Conclusion: Results of the present study suggest that BCSA can be used as a selective medium with high specificity for primary isolation and identification of Burkholderia cepacia complex bacteria.

Keywords: Keyword: Burkholderia cepacia, Isolation, Hospital, Infections.

P51: **Seroprevalence of tularemia in south eastern of Iran**

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Background and Aim: Francisella tularensis is etiologic agent of tularemia that is a zoonotic pathogen. Butchers and slaughterhouse workers are considered as an occupational group at risk of tularemia. The aim of this study was to assess the rate of tularemia seropositivity among butchers and slaughterhouse workers in southeastern of Iran.

Methods: In this study, 184 sera samples collected from butchers and slaughterhouse workers in 10 counties of Sistan and Baluchestan province. Sera were tested to detect specific IgG antibodies of tularemia using ELISA test.

Results: Seroprevalence of tularemia was 6.52% (95% CI: 3.58%-10.82%). There was no statistically different in seroprevalence of tularemia between butchers (5%) and slaughterhouse workers (9.38%). Tularemia seroprevalence in north part (9.30%) was more than the south (2.22%) and central (6.25%) parts of this province. The highest seroprevalence observed in Zabol (10.53%) and Nikshahr (10%) counties.

Conclusion: In this study, a relatively high seroprevalence of tularemia were observed among slaughterhouse workers and butchers in south eastern Iran. It is suggested that similar studies be done on other high risk groups, domestic and wild animals in this region to help the clarification of the epidemiological aspects of tularemia in south eastern Iran.

Keywords: Tularemia, Seroprevalence, Iran, Butcher, Slaughterhouse worker.

P52: **Seroprevalence of Q fever and brucellosis in Kurdistan**

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Background and Aim: Monitoring, control and prevention of zoonotic diseases is considered an important challenge for health systems worldwide. The aim of this study was to assay the seroprevalence of these two diseases in high risk populations of Kurdistan province in western Iran.

Methods: Two hundred fifty sera samples were collected from at risk individuals (include hunters and their families, butchers, health care workers and those referred to medical diagnostic laboratories) in the south west regions of Kurdistan province. Sera were tested to detect specific IgG antibodies by ELISA against brucellosis and Coxiella burnetii (Phase I and II).

Results: The seroprevalence of brucellosis and Q fever (C. burnetii IgG phase I and II) was 6.4% and 27.83% (20% and 14.52%), respectively. The highest seroprevalence of Q fever (38%) and brucellosis (12%) was seen in butchers. Age had a significant positive association with Q fever seropositivity (p=0.04). The seroprevalence of Q fever was higher in those people who had been in employment for more than 10 years (21.88%) compared to others (7.79%) (p=0.02). The keeping of animals (p=0.03), hunting and eating the meat of wild animals (p=0.02), and omitting to disinfect hands and faces after working (for health care workers and butchers) (p=0.02) were risk factors for Q fever seropositivity.

Conclusion: This study showed a relatively high seroprevalence of brucellosis and Q fever in high risk populations of Kurdistan province. It is suggested, that complementary studies be carried out in other parts of western Iran, to clarify the epidemiological aspects of these diseases.

Keywords: Coxiella burnetii, Brucellosis, Seroprevalence, Kurdistan, Risk factor.

P53: **The study of Silymarin effects after maternal exposure to Lipopolysaccharide on histopathological and immunological profiles in offspring**

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Background and Aim: It was suggested in numerous studies that the events occurring in preparturition period and the neonatal period is effective on subsequent disorders. Structural lipopolysaccharide (LPS) which is a part of gram negative bacterial cell wall, have been accepted as a model for studying bacterial infections and also an immune system stimulant and stressor. As indicated in many studies, silymarin has antioxidant and anti-inflammatory effects. It has been reported that the inflammatory conditions like asthma and infections during pregnancy are associated with the decrease in embryonic growth, increase of infection risk in neonates as well as undesirable health problems in adults. When reviewing scientific sources it was concluded that no coherent study was conducted up to now which analyses the protective and anti-inflammatory effects of silymarin on immune system of progeny against LPS and in reducing the degenerative effects of lipopolysaccharide during the embryonic period. The results of this research could be considered as an opening to future studies towards the better understanding of key factors involved with the fetus during pregnancy times.

Methods: To study the inflammatory conditions associated with the decrease in embryonic growth, increase of infection risk in neonates and the protective effects of silymarin, in this research, nine groups of mice were challenged with various doses of LPS and silymarin in a specified time period. IL-6, IL-1β and TNF-α indirect enzyme linked immunosorbent assay (ELISA) kits were used in order to study immunologic changes. Qualitative histological changes in liver and brain tissues were observed through a histopathological study on formalin fixed sample tissues.

Results: In the current research, maternal exposure to LPS caused a significant increase in immunological responses as well as brain and liver inflammation in offspring. Following the exposure to silymarin, the degenerative effects of LPS on brain and liver were extremely reduced.

Conclusion: The results proved protective effects of silymarin against LPS on brain and liver tissue and also immune system components. Silymarin reduced inflammation in nervous tissue which decelerated degenerative process. This phytogenic compound decreases hepatotoxicity and lipid peroxidation through various mechanisms, hence early intervention of silymarin prevents the adverse effects of immune system stressors on progeny. However, the results of this research can also be considered as an opening to future studies towards the better understanding of key factors involved with the fetus during pregnancy.

Keywords: Histopathology, IL-1β, IL-6, Lipopolysaccharide, Silymarin, TNF-α, Prenatal

P54: **First report and Detection of the KPC Gene in Pseudomonas aeruginosa clinical isolates from arak, iran with phenotypic test**

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Background and Aim: Detection of carbapenemase producers in the clinical laboratoryis of major importance for the determination of appropriatetherapeutic schemes and the implementation of infectioncontrol measures .The objective of this study was to determine the phenotypic characteristics of KPC-producing Pseudomonas aeruginosa isolates collected from hospitals in arak, iran.

Methods: A total of 100 Pseudomonas aeruginosa isolates samples were collected from hospitals in Arak, Iran. The antibiotic susceptibility testing was performed according to the Clinical and Laboratory Standards Institute's (CLSI) recommendations.then KPC determined by P. aeruginosa Modified Hodge Test(PAE-MHT), With replacing the indicator strain with Klebsiellapneumonia Sensitive to imipenem and meropenem .

Results: From the 100 collected isolates Pseudomonas aeruginosa. twenty-two/22 isolates were KPC positive evaluated by PAE-MHT showed the production of carbapenemases.

Conclusion: We now report there appears to be a high percentage KPC producing P. aeruginosa in Arak, Iran .Modified Hodge test is a simple test which can be performed to detect carbapenemases producing bacteria but the PAE-MHT with K. pneumonia Sensitive to imipenem and meropenem as the indicator strain can be performed in the routine lab for detection of P. aeruginosa isolates suspected of producing carbapenemases .

Keywords: Modified Hodge Test, KPC, Carbapenemase, imipenem

P55: **PCR method for detection of bacteremia caused by Enterococcus faecalis in vitro model**

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Background and Aim: Enterococcus faecalis is the third cause of nosocomial infections. Most of these infections usually are occurred by strains that are resistant to most antibacterial agents such as vancomycin. Bacteremia in adults and septicemia in children are most usual enterococcal diseases that need to be treated early and correctly . This, require rapid detection and characterization of bacteria in blood and determination of its antibiotic resistance type. Traditional method that is performed by isolation of the bacteria and identification by multiplex biological tests and determination of its antibiogram chart and MIC is usually time consuming and expensive and is not suitable for diagnosis of bacteremia. It must be substituted by more rapidly methods.

Methods: The aim of this study was evaluating a Multiplex-PCR assay for diagnosis of bacteremia due to Enterococcus faecalis and detection its resistance type to vancomycin by using primers specific to genus, species and resistance genes. We uased two standard strains resistant to vancomycin to prepare some suspensions with different bacterial contents for inoculation to defibrinated sheep blood samples for preparing blood samples with different bacterial content. PCR and routine method performed for all blood samples.

Results: Result of culture for all blood samples with ?5 CFU/ml was positive. We found van A in a strain and van B in two different standard strains by using a Multiplex-PCR with two pairs specific primers.

Conclusion: Regarding to potency of multiplex-PCR in simultaneous detection and identificationtion of the bacteria and its resistance pattern to vancomycin which is performable in one work day, and this affect treatment result obviously, it can be considered as a reliable and rapid alternative for routine method but it is required to increase its sensivity by using a more efficient commercial method for DNA extraction of bacteria in blood.

Keywords: Enterococcus faecalis, PCR, Microbial culture, Blood

P56: **Comparison of the RE and B1 gene for detection of Toxoplasma gondii infection in children with cancer**

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Background and Aim: Early, accurate and effective diagnosis of toxoplasmosis can make an important contribution to the prevention and control of disease, especially in people who are at risk. In this study, two commonly used genomic repeats of Toxoplasma gondii, RE (GenBank accession number AF146527) and B1, were compared to each other in nested-PCR assay.

Methods: Five hundred and thirty-five blood samples from children with leukemia were tested for the presence of T. gondii antibodies using enzyme immunoassays. One hundred and ten DNA samples of these patients (50 IgM+ IgG+, 10 IgM- IgG+ and 50 IgM-, IgG-) were analyzed by nested-PCR. The specificity of two nested PCR assays was determined using the DNA samples of other parasites and human chromosomal DNA.

Results: As a result, 82% (41/50) and 68% (34/50) of the IgM+, IgG+ samples were positive on duplicate RE and B1-nested PCR analyses, respectively. None of the 10 IgM-, IgG+ seropositive samples was detected positive after testing RE and B1-nested PCR assays in duplicate. One (2%) of the 50 seronegative samples was positive by duplicate RE-nested PCR but none of them were positive by duplicate B1-nested PCR. The detection limit of the RE-nested PCR assay was 640 fg of T. gondii DNA whereas this rate for B1-nested PCR was 5.12 pg of the DNA template. No cross-reactivity with the DNA of other parasites and human chromosomal DNA was found.

Conclusion: The results indicate that an RE-based nested PCR assay is more sensitive than B1 genomic target, of those tested, for detection of T. gondii. It is noteworthy that in comparison with B1-nested PCR, RE-nested PCR could detect the T. gondii DNA in seronegative samples too.

Keywords: RE, B1, Nested-PCR, Toxoplasma gondii, Children with cancer

P57: **Application of multiplex PCR for detection and differentiation of Entamoeba histolytica Endameba dispar and Endameba moshkovskii**

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Background and Aim: Entamoeba moshkovskii and Entamoeba dispar are impossible to differentiate microscopically from the pathogenic species Entamoeba histolytica. Multiplex polymerase chain reaction (Multiplex PCR) is a widespread molecular biology technique for amplification of multiple targets in a single PCR experiment.

Methods: For detection and differentiation of the three microscopy indistinguishable Entamoeba species in human, Multiplex PCR assay using different DNA extraction methods was developed. A conserved forward primer was derived from the middle of the small-subunit rRNA gene, and reverse primers were designed from signature sequences specific to each of these three Entamoeba species.

Results: A 166-bp PCR product with E. histolytica DNA , a 580-bp product with E. moshkovskii DNA and a 752-bp product with E. dispar DNA were generated.

Conclusion: We recommend this PCR assay as an accurate, rapid, and effective diagnostic method for the detection and discrimination of these three Entamoeba species in both routine diagnosis of amoebiasis and epidemiological surveys.

Keywords: Entamoeba histolytica, Entamoeba dispar, Entamoeba moshkovskii, Multiplex PCR, DNA Extraction

P58: **Extended-spectrum β-lactamase-producing Acinetobacter baumannii and genomic pattern by Pulsed-field gel electrophoresis**

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Background and Aim: Antibiotics such as fluoroquinolones are used for treating infections caused by Gram-negative bacteria, including Acinetobacter baumannii. A. baumannii strains some time have Extended-spectrum β-lactamase (ESBL) but ESBL production is rather rare. Resistance to fluoroquinolones antibiotics is mediated by lactamases and other mechanisms of resistance. The aim of the present study was to investigate of the prevalence of ESBL production and clonal relatedness of A. baumannii in Iran.

Methods: A. baumannii isolates identified from patients at hospitals in Kermanshah, Iran, were studied. The double disk method was used for detection of ESBL production. The susceptibility to different antibiotics was determined by the disk diffusion method (CLSI). Clonal relatedness was determined by pulsed-field gel electrophoresis (PFGE).

Results: This study showed high prevalence of resistance to Ampicillin and Cefpodoxime (98.1 and 92.3%). Fifty-two of the 104 isolates were identified as ESBL producers. Only colistin and tigecycline remained active against all isolates tested. The PFGE identified eight distinct pulsotypes: A (n=9), B (n=10), C (n=2), D (n=5), E (n=9), F (n=15), G (n=1) and H (n=1).

Conclusion: The clone F was dominant across different wards of the hospitals and appeared to be endemic. The level of antibiotic resistance was higher in clones A, B and F was higher than in others.

Keywords: Acinetobacter baumannii, β-lactamase, PFGE

P59: **Staphylococcus aureus nasal carriage determination in healthy adults at central Iran**

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Background and Aim: Staphylococcus aureus continues to be major cause human pathogens. In humans, S. aureus colonization is mainly found in the anterior nares and seems that 80% of strains causing bacteremia in carriers were endogenous. Three patterns of S.aureus carriage can be distinguished , include: persistent carriers ,intermittent carriers and non carriers. Therefore, the aim of this study is to determine the rate of Staphylococcus aureus nasal carriage in healthy adults

Methods:: A total of 813 healthy adults were subjected to this cross sectional study, from november2011 tojanuray2012 in Arak University of Medical Sciences. Samples were collected from both anterior nares of the volunteers by rotating a sterile Transwab with Amies clear ansport medium (MW170 Transwabs; Medical Wire and quipment Company, Corsham, UK). Different biochemical tests were performed on samples such as each of all the colony was inoculated onto sterilized phenol-red mannitol salt agar plates and incubated at 37 for 24 h. Isolates that were positive to catalase test, slide Coagulase and tube coagulase with human plasma and DNase test and thermostable nuclease were considered as S.aureus in this study . Also all isolates were checked for the presence of the sa442 gene(identification marker) by PCR.

Results: Among 813 isolates, 83 (10.2%) and 86(10.6%) were persistent carriers and transient carriers, respectively. 644 (79.2%) were non-carriers.

Conclusion: The total range of nasal carrier in this study is 20.8%, which is accordance with normal nasal carrierity in most part of the world. Although these range could be from 10%-70% in particular place, the carriage rate in the present study cannot be generalized because the sample population was a select community, comprising mainly students at a small facility. However, S.aureus nasal carriage is a cause for public-health concern .Therefore, it is necessary to take measures to control and reduce the rate of colonization. A more comprehensive study involving a larger population should be conducted to represent the Iranian population.

Keywords: Staphylococcus aureus ،nasal carriage, healthy adult

P60: **Comparative Prevalence Infection of Helicobacter pylori, Helicobacter hepaticus, Helicobacter bilis and Helicobacter pullorum in gallbladder of patients with and without Biliary Tract Diseases**

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Background and Aim: Cholelithiasis is one of the most common digestive surgical disorders, In addition, biliary diseases and number of gallstone, cases has gradually increased. Recently, several authors have reported that Helicobacter pylori and Bile tolerant helicobacter DNA has been found in human biliary tree and colonize the biliary tract in animals. The aim of this study is planned the present case control study to investigate To determine whether presence of Helicobacter species, particularly Helicobacter pylori, Helicobacter hepaticus, Helicobacter bilis and Helicobacter pullorum in gallstones, bile juice, and gallbladder mucosa of patients with biliary disease and bile juice of patients without biliary disease in Alzahra Hospital-Isfahan.
Methods: Serum and Bile juice of control subjects was collected from 25 patients without biliary diseases undergoing ERCP, and collected Serum, gallstones, bile juice, and gallbladder mucosa of 52 patient with biliary diseases undergoing laparoscopic cholecystectomy, Among them, 24 were diagnosed acute cholecystitis and 28 chronic cholecystitis. The three groups are comparable in age and sex composition. The presence of Helicobacter pylori in DNA extracted from these samples was performed using Helicobacter hsp60 gene specific primers, and presence of Helicobacter hepaticus, Helicobacter bilis and Helicobacter pullorum in DNA extracted from these samples was performed using species-specific 16S rRNA genes DNA was determined by polymerase chain reaction. The bile juice was culter in R broth and R slant (Described Richards CL et all) and sera were tested for presence of Helicobacter pylori-specific immunoglobulin G quantitatively by ELISA. The three groups are comparable in age, sex and BMI composition.
Results: 21 of 24(87.5%) patients with acute cholecystitis diseases and 25 of 28 (89.2%) patients with chronic cholecystitis diseases, and 20 of 25(80%) patients in the control group, showed increased level of Helicobacter pylori-specific immunoglobulin G. Helicobacter pylori DNA was detected in 6 of 24(25%) bile juice of acute cholecystitis and 2 0f 28(7%) bile juice of chronic cholecystitis, and 1 of 24(4.2%) gallbladder mucosa of acute cholecystitis and 1 of 28(3.5%) gallbladder mucosa of chronic cholecystitis. Helicobacter bilis DNA was detected in 1of 24(4.2%) bile juice of acute cholecystitis, and 1 of 28(3.5%) bile juice of chronic cholecystitis, respectively. No Helicobacter species were grown from the bile. All remaining DNA extraction samples were negative for Helicobacter hepaticus and Helicobacter pullorum. All samples were negative for H. pylori, H. hepaticus, H. bilis and H. pullorum in control patients. All gallstones DNA extraction samples were negative for Helicobacter DNA.
Conclusion: PCR technique can detect Helicobacter spp. DNA in gallbladder mucosa and bile of patients with chronic and acute cholecystitis without having a pathogenic role in hepatobiliary diseases. To clear the clinical role of Helicobacter spp. in the hepatobiliary diseases, we needed more studies on larger populations of patients and control groups to ascertain whether they have a role as a causative agent in the pathogenesis of biliary diseases. Thus, we investigated the presence of Helicobacter species in gallbladder mucosa and bile of Iranian patients.

Keywords: Helicobacter Pylori, Helicobacter Hepaticus, Helicobacter Bilis , Helicobacter pullorum, hsp60 gene, Cholecystitis,

P61: **Comparison of In vitro expression technology (IVET) and signature-tagged mutagenesis (STM) to identify important novel in-vivo target For Anti Infective chemotherapy, mini review**

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Background and Aim: The numbers of global infections produced by bacterial strains that are resistant to single and multiple antimicrobial drugs are on the rise. Concomitant with this alarming upward trend, there is a clear downward trend in the intent and determination of Pharmaceutical companies to develop novel antimicrobials. The emergence and increasing prevalence of bacterial strains that are resistant to available antibiotics Demand the discovery of new therapeutic approaches. So Too must efforts to identify novel agents and strategies for the Prevention and treatment of bacterial infection. Innovative Strategies are therefore needed to discover novel antibiotic Targets as well as alternatives to classical antibiotics. Bacterial virulence may therefore offer unique Opportunities to inhibit the establishment of infection or alter its Course as a method of antimicrobial chemotherapy. Targeting bacterial virulence is an alternative Approach to antimicrobial therapy that offers promising opportunities to inhibit pathogenesis and its Consequences without placing immediate life-or-death pressure on the target bacterium. Targeting bacterial virulence is an alternative approach to the development of new Antimicrobials that can be used to disarm pathogens in the host. The overall strategy is to inhibit specific mechanisms that promote infection and are essential to persistence in a Pathogenic cascade (for example: Toxin gene transcription, Quorum sensing, Secretion systems, Adhesion.)

Conclusion: IVET have been used in selection procedures to isolate genes that are preferentially expressed under In vivo compared with in vitro conditions. IVET couples the insertion of random Chromosomal segments upstream of an identifiable gene needed for survival in an animal model. IVET has been used to identify hundreds of in vivo expressed genes But suffers from only being able to identify genes that are highly expressed in vivo and also are not expressed In vitro. Advantages of IVET is down stream screening is minimized as induced genes are selected and no limitation in the number of clones that may be screened at once. Threshold level of induction must be reached to over come selection is Limitations of IVET and only induced gene are identified , not down regulated genes Limitations of IVET and Mutants must be generated in order to assess the role of the identified genes in virulence Limitations of IVET. STM is a high-throughput system based on mutagenesis due to random insertions that relies on negative selection in animal models of infection. STM and its derivatives have been used to identify genes essential for Colonization and infection for a wide variety of micro-Organisms. Advantages of STM is can be applied to multiple infection models to identify functions important for one infection or many and is a selection so is able to minimize the number of genes to study. Limitations of STM are In vitro essential genes will be excluded False-negatives can occur due to: clonal effects and competition with wild type clones in pool and complexity of pools of tags.

Keywords: Anti Infective chemotherapy, In vitro expression technology , signature-tagged mutagenesis

P62: **A novel class I integron-associated gene cassette in MDR Salmonella enterica serovars isolated from human**

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Background and Aim: In recent years multidrug-resistant Salmonella serovars have been isolated at an increasing rate. MDR in Salmonella serovars is strongly associated with class I integrons. In this study, we have investigated gene cassette contents of class I integrons among Salmonella serovars isolated from human clinical samples.

Methods: The 43 MDR Salmonella serovars which showed resistance to more than two antibiotic families by disk diffusion method included in the study. PCR assay were used to identify resistance genes and class I amplicons. Amplicons of class I integrons were sequenced.

Results: Thirty-eight (88/3%) of 43 MDR Salmonella isolates carried class I integrons. Integron- positive isolates showed six Salmonella serovars. Thirty-three integrons contained one gene cassette and belong to six serovars (Salmonella enterica serovars Enteritidis, Typhimurium, Paratyphi A, Paratyphi B, Paratyphi C and Havana. Five integrons contained two gene cassettes and belong to two serovars (Salmonella enterica serovars Enteritidis and Paratyphi C). A unique integron with the gene cassettes aadA2, blaPSE-1(1, 1.2 kb) was found in two Salmonella enterica serovar Enteritidis. ACCESSION JQ968458.

Conclusion: The integrons with aadA2 and blaPSE-1gene cassettes are very common among MDR Salmonella Typhimurium DT104. Similar gene cassettes have been identified in Salmonella enterica serovars Agona, Paratyphi B and Meleagriridis. To our Knowledge, this is the first report in the Enteritidis serovar and indicates that integrons- associated resistance genes may be exchanged between Salmonella serovars.

Keywords: MDR Salmonella, gene cassette, aadA2 and blaPSE-1

P63: **Methicillin resistant staphylococcus haemolyticus**

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Background and Aim: Staphylococcus haemolyticus is a coagulase negative member of the genus staphylococcus .the bacteria can be found on normal human skin flora.S.haemolyticus started emergin as major cause of nosocomial infections. Reported cases of infections caused by S.haemolyticus include septicemia, peritonitis, wound, UTI ,bone and joints.The ability of the bacteria to simultaneously resist against multiple types of antibiotic has been observed. one of the common antibiotics that are subject to resistance in S.heamolyticus is methicillin. The aim of this study was to determine the prevalence of methicillin resistant staphylococcus haemolyticus.

Methods: During one year study from june 2012 to june 2013 , 24 S.haemolyticus isolated from variety of clinical samples. The bacteria were identified through biochemical parameters such as haemolysis ,colony size, coagulase, novobiocin and polymyxine B susceptibility , ONPG, urease, hydrolysis and VP test.The antimicrobial susceptibility test performed by CLSI method using oxacillin, cefoxitin,trimethoprim,sulfamethoxazole, gentamycin, erythromycin,clindamycin,ciprofloxacin.Methicillin resistant were examined by both oxacillin and cefoxitin discs.

Results: From 24 clinically isolates, 13 cases were wound, 6 urine ,2 BALs, 1 eye discharge ,ear discharge, pleural fluid respectively.14 cases were methicillin resistant including: 6 wound ,6 urine, 1 BAL, pleural fluid and ear discharge respectively.18 patients were men and 6 were women.

Conclusion: Although staphylococcus haemolyticus is relatively less virulent than some other staphylococcus such as S.aureus, the ability of the species to acquir multi antibiotic resistance has made it a serious threat to world wide health care facilities .In some study in 2005 70% of S.haemolyticus isolate were methicillin resistant. In our study 57% were methicillin resistant, this finding indicate that isolation, identification and antimicrobial subtibility of s.haemolyticus shoud be significantly noted.

Keywords: staphylococcus haemolyticus. methicillin resistant. infection

P64: **Study of rpsl gene mutations related to resistance to streptomycin in clinical strains of mycobacterium tuberculosis**

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Background and Aim: Objectives: Streptomycin have been a major component of therapy for tuberculosis It is one of the most effective drugs for treatment of multi-drug Tuberculosis disease. Incidence of resistance is increasingly reported. . In this study, resistance rapid investigation to antibiotic streptomycin was conducted in clinical strains of Mycobacteriumtuberculosis. Streptomycin resistance is mainly related to mutation in gene “rpsl” codons 43 and 88 cause of drug resistance to streptomycin.

Methods: In this study 51 clinical isolates of Mycobacterium tuberculosis with positive culture and biochemical methods were used 34 strains of resistant and 17strain sensitive to medication were selected for possible mutations examination in gene rpsl. Specific primers used for performing PCRwere rPSL2 and rpsl 1. PCR products in a PCR-RFLP method by means of MboII endonuclease were examined. Some selected isolates were sent for sequencing to Source Bioscience company.

Results: Band504 bp was achieved by electrophoresis. From 51 isolates, 34 strains were resistant and 17 ones were susceptible phenotypically. As a result of molecular method, all of susceptible isolates were non mutant by PCR-RFLP method. In the other hand,eh t tsom of all resistant strains have mutation in codon 43 . Results of sequencing method were proved the results of molecular method

Conclusion: The results of this study indicate that the PCR-RFLP method can be used in routine work as a simple and rapid method for detection of resistance to Streptomycin.

Keywords: Mycobacterium tuberculosis, drug resistance, Streptomycin , PCR-RFLP

P65: **isolation of ecoli o157: H7 special lytic bacteriophages of feedlot cattle for remove ecoli o157: H7 bacterial infection in mouse**

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Background and Aim: Among the various hemorrhagic E.coli races,E.coliO157: H7 is the commonest verotoxin producer serotype.This poison causes inflammation , intestinal bleeding and severe diarrhea along with hemolytic uremic syndrome(HUS) and kidney insufficiency. Regarding microbial resistance against different antibiotics,finding new treatments to diseases have always been researchers' concern. Since bacteriophages are generally the special to each bacterium , they can be used to remove bacteria as well as to treat infection . The purpose of this study was to isolate special phages of E.coli O157: H7 bacterium from cows' sewage and applying it as antibacterial factor to treat infection caused a by this bacterium.

Methods: To isolate E.coli O157: H7 bacterium from sorbitol mac conkey agar and eosin methylene blue(EMB) and IMVIC sepration medium and special anti serum E.coli O157: H7 has been used.To isolate special lytic phages,the researcher passes the cows' sewage 3500×g centrifuged for 25 minutes then the gained supernatant is passed through the 0/45µ biological filter afterward the nutrient broth and E.coil O 157: H7 are added to it,the sample is put in 150 rpm shaker incubator at 37 degree temperature for 18 hours then it is recentrifuged and passed through 0/45µ filter and the filterized liquid was analyzed in the following three ways1.Double overlay technique 2.Disk sensitivity test 3.creating holes. and at the animal model stage the effect of isolated bacteriophages on mouse bacterial infection was observed.

Results: Findings show that from the 70 isolated sample ,19 phages samples were recognized having special lytic activity against E.coli O 157: H7.In this research , three species of standard non-hemorrhagic such as EIEC, ETEC, and EPEC , as indicators , were used. Isolated lytic phages caused removing the infection of E.coli O157: H7 of four groups of mice.

Conclusion: It is concluded that the lytic bacteriophages have the ability to replace antibiotics.

Keywords: 1-lytic bacteriophage.2-ecoli o157: H7.3-sewage.4-mouse.

P66: **Prevalence of Clindamycin and Erythromycin resistance phenotypes in staphylococcus aureus clinical isolates by D test**

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Background and Aim: in this study, the phenotypes of resistance to Clindamycin and Erythromycin; especially inducible resistance to Clindamycin in S.aureus was surveyed; also antibiotic susceptibility between Methicillin susceptible and resistant S.aureus (MSSA and MRSA) strains was compared, and MRSA strains were detected by phenotypic and genotypic tests. S. aureus is one of the most infectious opportunistic nosocomial bacteria. The range of clinical manifestations varies widely, from skin surface lesions, to systemic and fatal infections. Antibiotic resistance has come as a concerning issue in laboratory and clinical settings; especially MRSA strains that are multi resistant. Moreover inducible resistance to antibiotics such as clindamycin is important, because of failure in diagnosing it in common laboratory tests and different results between in vivo and in vitro settings. This phenotype is one of several states that may be occur in the presence of erythromycin in vitro, or prior to clindamycin consumption. Several other phenotypes have been reported in respect to clindamycin and erythromycin resistance, including, R (resistance to both), S (susceptible to both), D+ (colonies in clindamycin susceptibility zone), Hazy zone of clindamycin and Negative phenotype.

Methods: Among 209 S.aureus hospital isolates from different clinical origins, such as trachea, blood culture, skin and tissue culture, the identification tests including DNase, mannitol fermentation and coagulase tests were performed. Then antibiogram test was done after preparing half Mc farland and culture on Muller Hinton Agar (MHA) plate by Kirby Bauer assay according to CLSI. Standard S.aureus strain (ATCC25923) was tested and adjusted. D- test was performed in this test on the same media and phenotypes distinguished. To detect MRSA, oxacillin disc was used and then, the results confirmed after detecting mecA gene.

Results: Resistance to amoxicillin was 80/5 percent. All isolates were susceptible to vancomycin. Antibiotic resistance was significantly higher in MRSA than MSSA. Resistance to other antibiotics was: erythromycin (27%), clindamycin (22%), co-trimoxazole (11%), tetracycline (43%), ciprofloxacin (31%), gentamycin (19%) and oxacillin (32%). 21% of isolates were resistant to all antibiotics, except to vancomycin, co- trimoxazole and gentamycin. Inducible resistance to clindamycin was 4%. R and S phenotypes were 50% and 15%, respectively. D+ phenotype was 2%; while HD and Negative ones were not observed. Detection of MRSA performed by phenotypic and genotypic tests was 32%.

Conclusion: Antibiotic resistance in bacteria is an increasing problem. S. aureus can resist antibiotics by different ways. MRSA strains are more resistant and virulent in special individuals. Antibiogram test helps the pattern of susceptibility and probability of being up to date by treatment as some strains of S. aureus has been reported as multi drug resistant. Inducible resistance to clindamycin occurs in vivo, while common laboratory tests do not detect it unless by D- test. This phenomenon is not high in percentage, but can help to consumption of more efficient antibiotics.

Keywords: Staphylococcus aureus, MRSA, MSSA, D-test, inducible resistance,clindamycin

P67: **Agr and sccmec types in Staphylococcus aureus clinical isolates from tracheal origin and a comparison between Methicillin susceptible and resistant strains.**

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Background and Aim: Staphylococcus aureus can cause respiratory tract infections in addition to a variety of clinical manifestations attributed to it, especially Methicillin resistant s.aureus has more ability to transmit and resist a variety of antibiotics by producing Penicillin Binding Protein2a (PBP2a) that has lower affinity to beta- lactam antibiotics. sccmec types (I- VIII) encode this enzyme by mecA gene staphylococcal chromosome. MRSA strains cause necrotic pneumonia especially after viral infections (such as Flu) in respiratory system. These strains are more pathogenic than Methicillin susceptible strains. Also, agr types (I-IV) have a major role on regulating staphylococcal virulence by producing auto-inducer peptide (AIP). This study was conducted to find out the prevalence of agr and sccmec types in S. aureus clinical isolates taken from tracheal samples of patients with respiratory disorders.

Methods: Among 100 tracheal samples from patients the identification tests by catalase, slide and tube coagulases, acid production from mannitol on mannitol salt agar, DNase tests and colony morphology on blood agar, were performed. Then, the isolates preserved in Tryptic Soy Broth with 30% glycerol in -70□C. DNA extraction performed by lysostaphin protocol. The polymerase chain reaction (PCR) was done to detect the prevalence of mecA gene and subsequently sccmec and agr types in the isolates.

Results: Methicillin resistance was confirmed by detection of mecA in the isolates. Methicillin resistance rate was 22%. Among sccmec types, type 3 was detected in 80% of isolates. sccmec types 1 and 5 were found in 2.5% and 17.5% of isolates, respectively. Types 2 and 4 were not found. The agr types prevalence for type I was 63% and for types II, III and IV was 21%, 12% and 11%, respectively. The agr types in MRSA were: type 1 in 44% and types 2, 3 and 4, were 23%, 15.5% and 7.5%, respectively. Also, in MSSA strains, agr types 1, 2, 3 and 4 were 49%, 21%, 18% and 12%, respectively.

Conclusion: Methicillin resistance may vary according to clinical origin and was low in tracheal samples. Sccmec type 3 is the most type in Iran and then, the most MRSA strains are hospital- acquired. Also, agr type1 is the most prevalent type in both MRSA and MSSA strains.

Keywords: MRSA, respiratory tract, sccmec, agr types

P68: **Multi- drug resistant Hospital –Acquired Methicillin resistant staphylococcus aureus clinical isolates from blood culture of patients with bacteremia**

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Background and Aim: S.aureus is an opportunistic bacterium with ability to cause a wide spectrum of manifestations such as bacteremia and internal organs infections. In this respect, methicillin resistant s.aureus (MRSA) strains are more virulent and have been armed to resist a variety of antibiotics, in addition to beta lactam antibiotics. Hospital - acquired (associated) MRSA strains are resistant to a wide spectrum of antibiotics such as tetracycline, erythromycin, clindamycin, amoxicillin, ciprofloxacin, aminoglycosides and in sporadic cases reported to vancomycin. This is an important issue in increasing antibiotic resistance of these strains, and it may be difficult to remedy the infections in the future. Also, these strains have sccmec type 3 with ability to resist mercury. Nowadays, vancomycin is of some restricted remained antibiotics against staphylococcal infections. This study was done to evaluate the amount of antibiotic resistance among MRSA blood samples and also detect the multi-drug resistant isolates.

Methods: At first, 150 clinical isolates were identified by coagulase, mannitol fermentation, DNase and β- hemolysis on blood agar. Then antibiogram test was done by Kirby Bauer assay on Muller Hinton Agar medium according to CLSI (2012). After direct colony inoculum preparation, the antibiotic discs used were amoxicillin, ciprofloxacin, gentamycin, erythromycin, co- trimoxazole, oxacillin, clindamycin, vancomycin and tetracycline. Methicillin resistance was assessed by 1 µg oxacillin disk. The plates incubated for 18 – 22 h. zone of ≤10mm defined MRSA and ≥ 13mm was MSSA. 15 methicillin resistant blood sample clinical isolates were adopted. Genomic DNA was extracted according to lysostaphin method and sccmec types were assessed by polymerase chain reaction.

Results: antibiotic resistance among the MRSA isolates was various. 5 (33% of) isolates were resistant to all antibiotics, except to vancomycin and 4 (26.6% of) isolates were susceptible to co- trimoxazole and vancomycin only. 2 (13.3% of) isolates were susceptible to gentamycin, clindamycin and vancomycin, and 4 isolates were susceptible to gentamycin and vancomycin. Also, all the isolates Carried sccmec type 3.

Conclusion: Antibiotic resistance in HA-MRSA strains is high and also susceptibility of these strains to vancomycin is wide, too. In addition, co- trimoxazole, gentamycin and clindamycin are effective antibiotics against most of MRSA strains. sccmec type 3 is prevalent in MRSA strains and encodes factors resistant to a wide variety of antibiotics.

Keywords: multi-drug resistance, HA- MRSA, sccmec type 3,

P69: **Carbapenem resistant Acinetobacter baumannii carrying the ISAba1- associated blaOXA-23 and blaOXA-51 genes in Vali-Asr hospital ,Arak,Iran**

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Background and Aim: A.baumannii resistant to carbapenems has been isolated in Europe, Asia, North and South America and the emergence of carbapenem resistance in A. baumannii has become of global concern. The aim of this study was to describe the presence of the ISAba1 associated with blaOXA-23 and blaOXA-51 genes in A. baumannii isolates fromcentral teaching hospital in Arak,Iran.

Methods: A total of 63 non-duplicate A. baumannii isolates were collected from clinical and environmental specimens in the Valiasr hospital in the central province of Iran (March to September, 2011). The antimicrobial susceptibility for 23 antibiotics was determined by disk diffusion. Primers were used to amplify genes blaOXA-23-like, blaOXA-24-like, blaOXA-51-like and blaOXA-58-like. ISAba1was detected using primers ISAba1F and ISAba1R.Whether ISAba1 preceded the OXA carbapenemase genes was determined using PCR mapping experiments by combinations of the ISAba1F and reverse primers designed for the blaOXA-23-like and blaOXA-51-like genes

Results: Almost all isolates were resistant to a wide range of antimicrobials; the lowest resistance rate was found for colistin (11 %). The blaOXA-51-like and blaOXA-23-like genes and ISAba1 element were identified in all A. baumannii isolates. 27 isolates (42.8%) out of the 63 strains analyzed carried blaOXA-24-like gene. However, the blaOXA-58-like gene was not found in this study.54 and 16 isolates out of the 63 isolates were positive for ISAba1/ blaOXA-23-like and ISAba1/ blaOXA-51-like genes respectively

Conclusion: ISAba1 element may have an important role in the expression of blaOXA-23-like and blaOXA-51-like genes. High prevalence of ISAba1/ blaOXA-23-like in our isolates, suggest that this unit is mobile and plays important role in the development of carbapenem resistant A.baumannii isolates in our site.

Keywords: A.baumannii, OXA-type carbapenemases, ISAba1

P70: **High prevalence of methicillin resistant coagulase negative Staphylococci in clinical isolates of a tertiary hospital**

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Background and Aim: Coagulase-negative staphylococci (CoNS) have become increasingly recognized as important agents of nosocomial infection. Methicillin resistance Coagulase-negative staphylococci MR-CoNS may act as a source of Staphylococcal cassette chromosome mec (SCCmec) for Methicillin-resistant Staphylococcus aureus (MRSA). The frequency of MR-CoNS in health care–associated CoNS is currently increasing. The aim of present study is to investigate the prevalence and significance of MR-Cons isolated from clinical isolates at the University Hospital of the Vali-asr during a six-month period.

Methods: A total of 110 CoNS isolated from different clinical isolates from May2012 to November 2012 in Vali-asr hospital(Arak University of Medical Sciences) were subjected to this cross sectional study. Isolated strains were identified by using conventional methods (growth on mannitol salt agar, colony morphology), Gram stain, catalase- coagulase , DNAse tests, Acid production from mannose- xylose- maltose- sucrose, and urease test. All the samples were checked for methicillin suceptility by cefoxitin disk

Results: Of 110 isolate 70 MR-Cons were identified according to standard methods. Thses isolates. The strains were collected different clinical specimens, including blood 26(37?%), wound 12(17?%), urine 19(27?%), catheter tips 5(7.1?%), drainage fluids (5.4?%), sputum 2(2.85?%), and secretions in general 6(8.6?%).

Conclusion: A high prevalence of MR-CNS was found in clinical isolates. This is of concern in view of potential spread of mecA to S. aureus (MRSA). Multiresistant CNS strains might become an emerging problem for hospital setting. Further study is needed to investigate the dissemination of MR-CoNS in the community

Keywords: Coagulase negative Staphylococci- Methicillin-resistant- nosocomial infection

P71: **Detection of Mycolasma Hominis and Ureaplasma Urealyticum by Culture and Polymerase Chain Reaction (PCR) Procedures in Women With septic Abortion**

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Background and Aim: Mycoplasma hominis and Ureaplasma urealyticum can be isolated with considerable frequency from the human urogenital tract and are thought to cause various syndromes. Although M. hominis and U. urealyticum species can be cultured, this requires technical skill for interpretation of microscopic colonies and takes two to five days. The objective is to investigate the presence of mycoplasmas in women with genital infections due to abortions with culture and PCR method.

Methods: Appropriate samples(Blood or genital specimen) were collected from women with genital infections in Karaj's hospitals. Each sample was divided into two parts. The first was cultivated on specific media for mycoplasmas(Mycoplasma hominis and Ureaplasma urealyticum) and the second frozen for subsequent study by polymerase chain reaction (PCR). For Mycoplasma hominis culture , Samples were placed into arginine broth, and for Ureaplasma urealyticum, Samples were placed into urea broth .Each cultured samples were incubated at 35°C, and monitored four times daily for up to five days. Then the PCR test was performed.

Results: A total of 87 samples were studied.result showed that 57.9 % of the 20-30 aged patients had precedent abortion. From the total of cultured specimens, 12 cases were positive: 41.7 for Mycoplasma hominis, 16.7% for Ureaplasma urealyticum and 41.7 % for other bacteria. with PCR method 25 cases(33.33%) showed positive results: 60.0% for Mycoplasma hominis and 40.0% for Ureaplasma urealyticum. Result showed a meaningful statistical relation between PCR test results with recurrent abortion and level of education ($p< 0.05$).

Conclusion: Because of difficulties in culture method for detecting of mycoplasmas, our results showed that PCR is a more sensitive, easier and faster method in comparison to culture for detecting of bacterial cause genital infections due to septic abortions.

Keywords: Genital infections, Mycoplasma hominis, Ureaplasma urealyticum, culture method , Polymerase Chain Reaction

P72: **Frequency of class I and II integrons in clinical isolates of Klebsiella pneumonia producing bla-TEM, bla-SHV and bla-CTXM betalactamases in Zanjan**

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Background and Aim: Klebsiella pneumoniae is a common nosocomial pathogen which has been associated with various infections. Extended spectrum beta lactamases (ESBLs) of the TEM, SHV or CTX-M type confer resistance to many betalactam antibiotics. Class I and II integrons have an important role in resistance to multiple classes of antibacterial drugs.The aim of the present study was to determine the prevalence of blaTEM, blaSHV and bla CTX-M betalactamases and integron elements in Klebsiella pneumonia isolates in Zanjan

Methods: In this cross sectional study, a total 150 isolates of Klebsiella pneumoniae were collected from clinical samples such as urine, stool, wound and blood from 2012 to 2013 in zanjan hospitals. After verifying of isolatets by biochemical tests, Antimicrobial susceptibility testing was performed by disk diffusion method as recommended by CLSI guidelines .The presence of blaTEM , blSHV, bla CTX-M and integron genes was investigated by PCR using specific primers

Results: The most prevalent resistance profile was Erythromycin 99.3% (148 isolates), followed by Amoxicillin 95.3% (142 isolates) , Coamoxiclav 51.5% (76 isolates) , Aztreonam 51.5% (76 isolates), cefotaxime 41.6% (62 isolates) , ceftazidime 39.5% (59 isolates), Cefoxitine 38.9 % (58 isolates) , cotrimoxazole 36.9% (55 isolates) , ciprofloxacin 33.5% (50 isolates) , Tetracycline 29.5% (44 isolates), Amikacine 26.8% (40 isolates). Imipenem was found as an effectiveness antibiotic with susceptible rate 92%. Of this isolates, 62.41% were resistance to three or more agents and considered Multidrug resistant (MDR). Among 58 ESBL producing isolates, percent recovery of blaTEM , blaSHV and blaCTX-M was 63.7% (37 isolates), 86.2% (50 isolates) and 93.1% (54 isolates) , respectively. Class I integrons being observed in 79.3% (46 isolates) of clinical isolates, whilest 43.1% (25 isolates) of isolates contained class II integrons.

Conclusion: Our findings show high prevalence of Klebsiella pneumoniae producing beta-lactamases and class I integron in zanjan hospitals. Also, this structure is playing an important role in the development of multidrug resistance (MDR) in these strains.

Keywords: Integron, Klebsiella pneumonia, Extended spectrum beta lactamase

P73: **Determining of the bla CTX-M and bla TEM genes in ESBL producers of E. coli strains in Rasht-Iran**

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Background and Aim: Nosocomial infections are hygienic problems. These infections are caused by different microorganisms. Escherichia coli is one of the most common etiologic agent of nosocomial infections. Appropriate treatment of infections caused by E. coli with β-lactam antibiotics is important in cost and mortality reduction. Several factors such as extensive use of antibiotics in empirical treatment of infections caused by these bacteria and self treatment have resulted in the development of antibiotics resistance and treatment failure. The production of β-lactamases is the most prevalent mechanism responsible for the resistance to β-lactams. ESBLs producers are those strains of the Entrobacteriaceae which encode β-lactamases. These enzymes catalyze the hydrolysis of the amide bond in the β-lactam ring and confer broad resistance to penicillin's, aztreonam, cephalosporins (an oxyimino-cephalosporin) and carbapenems. ESBLs are often plasmid mediated and most of them are mutants of the classic TEM and SHV type enzymes. This study was conducted to determine the relative frequency of the presence of bla CTX-M and bla TEM genes in the ESBLs producing E. coli strains isolated from Rasht hospitalized patients.

Methods: In this cross-sectional study, 160 E. coli strains isolated from clinical samples including urine, ascitic fluid, secretions, wound and blood from hospitalized patients in Rasht hospitals from July to December 2012. Bacteria were cultured in Blood agar and EMB agar mediums. Biochemical tests were performed for confirmation of clinical isolates as E.coli. Antimicrobial susceptibility tests performed according to CLSI guidelines by disk diffusion method (Kirby-Bauyer) on Mueller-Hinton agar. ESBL production confirmed by Double disk assay with disks containing Ceftazidime (30µg), Ceftazidime (30 µg)/ ClavulanicAcid (10 µg) and Ceftriaxone (30 µg/Co-amoxiclav (30 µg). The plasmid DNA from ESBL-producing strains was extracted and blaCTX-M and blaTEM were detected using specific primers by PCR.

Results: Among 160 clinical isolates of E.coli collected from six hospitals in Rasht, the highest resistance was for Cephalothin (100%) followed by Amoxicillin, Cefotaxime and Ceftriaxone (98.8 %). All isolates were susceptible to Imipenem. Majority of the isolated E. coli (51.9%) were ESBL producers from which (86.7%) and (32.9%) had bla CTX-M and bla TEM genes respectively. None of these genes were found in 9.4% of the isolates.

Conclusion: Half of strains were able to produce ESBLs. This is a threat to healthcare systems in most cases and leads to treatment failure. It is also found that some of the resistant isolates had none of the blaCTX-M and blaTEM genes. Therefore one must look for other genes which confer ESBL production.

Keywords: β-lactams, ESBLs, E. coli, bla CTX-M, bla TEM

P74: **Consideration of intestinal protozoan parasites infections in day-care center childern in Karaj city in 2012**

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Background and Aim: Diseases caused by parasites are one of the most important economic- health problems of the most developing countries. Children who belong to very important constituents of society are at increased susceptibility to such diseases. In centers like kindergartens where people live collectively and interact closely, transmission of parasitic disease becomes ever more important. Constant and regular study in developing countries for planning to control these diseases is essential.

Methods: This is a descriptive study and sampling was random clustering from 154 active kindergartens of 9 districts of Karaj. In this study the prevalence of enterobius and other intestinal parasites in 904 children from the age of one to six years in Karaj in 1391 was studied. In order to collect the required data for example age and gender of the child and gender, age, occupation and education of the parents and effective factors on infection with intestinal parasites like hand washing and using personal drinking glass and clinical symptoms in children and symptoms reported by the child to his/her parents or caregiver were studied. in order to determine the demographic data, relevant questionnaires were distributed and at the end of the questionnaire the result of the scotch test, either positive or negative, was recorded.Formol ether and direct test were performed on three samples that were collected inconsecutively. For the eneterobius test, the scotch test which is more specific was used.

Results: In this study the number children were 904 and 460 of them (50.9%) were male and 444 (49.1%) were female. The overall intestinal parasite infection in formol ether test was 16.7% and in Scotch tape test for Enterobiusvermicularis was 2.3%. The most common protozoan was Blastocystishominis in 84 children (9.3%) and Giardia in 66 children (7.3%). Additionally, infection with Endolimax nana was reported in 3 children (0.3%) and Entamoebahistoletica was reported in 4 children (0.4%). In this study there was a significant correlation between intestinal parasite and children.

Conclusion: According to the findings of this study, the prevalence of infection to intestinal parasites especially giardia and blastocystis in kindergartens of Karaj is high. The reason for this could be due to lack of knowledge of children and their parents of the transmission methods and absence of treatment of infected and carrier people who act as healthy carriers. The fact that stool test is not performed before the children entering kindergartens and thatinfection with parasite is not diagnosed through laboratories in terms of frequency of parasite shedding in stool of carrier or infected people and finally the diagnostic techniques are not appropriate, the prevalence of infection in this group of children is high. Overall, inattention to personal hygiene, transmission of infection from mother to child and from child to child in studied kindergartens are effective in persistence of the disease. Therefore education of children, caregivers and parents on hygiene and effective treatment of infected people are keys to reduce the rate of infection and transmission.

Keywords: Intestinal parasite, Day-care centre, Child, Karaj

P75: **Epidemiological survey of animal bites in Golestan province, North of Iran, 2011- 2012**

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Background and Aim: Animal bite is a major public health problem because of its rabies risk and economic burden. Due to the high number of animal bites in the province, this study was to evaluate the incidence of animal bites, biting animal and the incidence of rabies.

Methods: In this studyfrom April 2011 to April 2012, available information based on the offices Animal Bites was collected, containing type of animal, gender, occupation and residency.

Results: The total number of individuals that exposed to animal bites was 10810. The mostly affected individuals were males (75/48%), in age group of less than 25(49.97%) and in rural residents (82/27%).Students (21.92%) and housewives (15.93%) had the highest percent of animal bites. In this study reported that one case was dead.

Conclusion: Due to the high level of animal bites in province, it seems that target groupeducation, including children, teenagers and animal owners plays an important role in decreasing of animal bites. The cost to the health care system of any animal bite is huge, preventive measures to avoid bites should be a priority.

Keywords: epidemiology, rabidity, animal bites

P76: **Comparison the distribution of MexAB-oprM gene among P.aeruginosa isolates from burnt patients to cystic fibrosis patients**

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Background and Aim: Infection by Pseudomonas aeruginosa (P.a) play an important role in endangering health of burned patient and cystic fibrosis. Beside, it's intrinsic and acquired resistant to common antibiotics like 3th generation of cephalosporins and carbapenems causes failure treatment. Therefore, using fluoroquinolons in it's eradication is very important. However, emerging of resistant strains even to fluoroquinolons is increasing recently. Several chromosomal tripartite efflux pumps play an important role on both intrinsic and acquired resistant in these bacteria. Of tripartite efflux pumps, the role of Mex-AB-oprM is more important. According to the above information the aim of this study was to determine the resistant profile to common antibiotics by emphasize on fluoroquinolons among Pseudomonas aeruginosa strains isolated from burnt patient and cystic fibrosis by molecular and phenotypic methods.

Methods: Infection by Pseudomonas aeruginosa (P.a) play an important role in endangering health of burned patient and cystic fibrosis. Beside, it's intrinsic and acquired resistant to common antibiotics like 3th generation of cephalosporins and carbapenems causes failure treatment. Therefore, using fluoroquinolons in it's eradication is very important. However, emerging of resistant strains even to fluoroquinolons is increasing recently. Several chromosomal tripartite efflux pumps play an important role on both intrinsic and acquired resistant in these bacteria. Of tripartite efflux pumps, the role of Mex-AB-oprM is more important. According to the above information the aim of this study was to determine the resistant profile to common antibiotics by emphasize on fluoroquinolons among Pseudomonas aeruginosa strains isolated from burnt patient and cystic fibrosis by molecular and phenotypic methods.

Results: Based on antibiogram test, resistant to imipenem and meropenem in burnt isolates were 82.5% and 53.8% respectively, in comparison to resistant of cystic fibrosis isolates to imipenem 12.5% and meropenem 15% respectively. By E- test, resistant to ciprofloxacin in burnt patient was 86.3% vs. to 32.5% in cystic fibrosis patients.

Conclusion: Existence of Mex-AB-oprM gene in 67.5% of Pseudomonas aeruginosa burnt strains and 55% of Cf strains in one hand and existence of high resistant to levofloxacin (90%) and ciprofloxacin (86.3%) vs to 40% and 37.5% resistance to Ciprofloxacin and Levofloxacin respectivelyamong cystic fibrosis isolates, distribution of Mex-AB-oprM gene was detected in 67.5% of burnt strains in comparison to 55% of cystic fibrosis P.aeruginosa strains in the other hand, shows the important role of efflux pumps in multidrug resistant Pseudomonas aeruginosa strains .Study of other resistant mechanisms is recommended in the future.

Keywords: Pseudomonas aeroginosa, efflux pump, cystic fibrosis

P77: **Isolation of symbiotic bacterium associated with pederin biosynthesis and pederin extraction from paederus beetle**

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Background and Aim: Amide toxin of pederin is produced by symbiosis bacterium and paederus beetle. Pederin could destroy eukaryotic cells by inhibition of protein synthesis and mitotic division. Hence, it is considered as an appropriate antitumor in cancer studies. The purpose of this study was pederin extraction and isolation of symbiotic bacteria.

Methods: After collection of paederus beetles, abdominal section was completely crushed then cultured in Pseudomonas agar. Pederin production by beetles was examined with linear dermatitis due to contact of crushed bodies of insects on the skin of rats. Also thin-layer chromatography was used for extraction of pederin.

Results: Colonies in selective medium were large, mucoid and produced green pigment. After Gram staining, the isolated bacterium was rod shaped and Gram negative. The results of morphological and biochemical tests showed that the bacteria was belong to the genus Pseudomonas sp. The extracted pederin was able to create linear dermatitis as swelling and irritated skin. A point with Rf=0.22 on thin-layer chromatography plate was confirmed the presence of pederin.

Conclusion: The results of this study showed that hemolymph of Paederus fuscipes collected from the province of Mazandaran contained pederin. In addition, the bacterium was cultivable. Culture of bacteria with pederin synthesis genes could be promising pederin production in large-scale.

Keywords: Paederus fuscipes; pederin; Pseudomonas

P78: **What determines clinical nature of Staphylococcus aureus: Molecular analysis of the Accessory Gene Regulator (agr) Locus and enterotoxin genes**

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Background and Aim: Although a relatively unspectacular, Staphylococcus aureus is a dangerous human pathogen, carrying a wealth of pathogenic determinants by which it is able to survive and invades into a variety of nonprofessional phagocytes. To do so, S.aureus produces a wide variety of toxic proteins, including the staphylococcal enterotoxins (SEs) A-E and G-J, and others. Each of these toxins is known to have potent effects on cells of the immune system, but many of them have other biological effects as well. These adhesins are influenced by an accessory gene regulatory locus named agr, which controls the balance of virulence factor expression in the colonization and invasion phases of infection. In the past decade, numerous molecular techniques have proved useful in identification and comparison of S. aureus isolates in epidemiological studies. However, very few studies have identified S. aureus isolates by the gene polymorphisms among important virulence-related genes. Secondly, associations between the agr genotype of isolates and specific staphylococcal diseases have been shown for toxic shock syndrome, staphylococcal scalded skin syndrome and abscesses and soft tissue infections; however, other clinical infections have not been studied in detail. The present study aimed to study S.aureus isolates for epidemiological analysis, using PCR-based techniques to i) gain insight into enterotoxins of the organism ii) to group these isolates by agr typing and ii) for linkage between agr groups and various clinical infections.
Methods: S.aureus isolated from various clinical infections in patients admitted to the University Teaching Hospital (Sina), between years 1382- 1388 and stored were selected by random sampling for this study. For confirmation of species and detecting meticillin resistance a multiplex PCR was carried out for genes nuc and mecA. The antibiogram was performed using disc agar diffusion method. DNA extraction was carried out using CinnaGen DNG kit with a slight modification. A set of six multiplex PCR tests were performed on each strain for the SEs and agr types, following which the products were analyzed by gel electrophoresis.
Results: All 151 S.aureus strains produced a clear PCR product of the expected size (279 bp), confirming the speciation of isolates and 54 of them were found methicillin resistant in view of harboring mecA gene. 32.5% of the strains were observed to be multi- drug resistant. Out of 151 strains, 91% were positive for at least one SEg gene, the most prevalent being sea (57.6%) followed by sek (51%) and seq (45.7%). 14 strains were negative for any SE genes. The most common toxin genotype in MRSA isolates was sea-sek-seq , followed by sek-seq while in MSSA sea sec sek seq was the prevalent one. Out of total isolates studied, 95.4% were positive for agr types; the most prevalent type was agr type 1(76.2%). Genotype sek, seq was mainly associated with agr type I.
Conclusion: A remarkable variation in the SEs genes depending upon methicillin status, year of isolation and source of S.aureus was found in this study.

Keywords: S. aureus, Enterotoxins, Epidemiology, MRSA, agr locus

P79: **Effect of zinc oxide nanoparticles on the biofilm formation by Pseudomonas aeruginosa**

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Background and Aim: Bacterial biofilm is a functional consortium of attached bacteria growing on animate or inanimate surfaces which are embedded in a polysaccharide matrix. Biofilm formation and their high resistance causes many relentless and chronic bacterial infections. Different approaches have been used for preventing biofilm related infections. This study aims at effecting of zinc oxide nanoparticles on the biofilm formation by P. aeroginosa.

Methods: Zinc oxide nanoparticles were sentisized by sol gel method. To determine the efficacy of ZnO nanoparticles in elimination of formed biofilm, tissue culture plate medium (TCP) method was carried out with suitable modifications. Individual wells of sterile, polystyrene, 96-well-flat tissue culture plates were filled with BHI broth, overnight culture of bacteria and ZnO nanoparticles. The tissue culture plates were incubated for 24 h at 37◦C. After incubation, the wells were washed and dried, respectively. Then 95% ethanol was added to the wells and the optical densities (OD) of stained adherent bacteria were determined with a microplate reader (model stat fax 3200) at 492 nm.

Results: ZnO nanoparticles showed potential anti-biofilm activity on P. aeroginosa bacteria that was tested in vitro on biofilms formed during 24 hours treatment. Treating these organisms with ZnO nanoparticles with concentration of 250 nM resulted in more than 80% inhibition in biofilm formation.

Conclusion: The present study characterizes the anti-biofilm activity of ZnO nanoparticles against P. aeroginosa that is been proven for their efficient biofilm formation. Observations made through microtiter plate assay (0.1% crystal violet staining) discloses the potential of ZnO nanoparticles in effective inhibition of biofilm formation.

Keywords: ZnO nanoparticles, Biofilm, Pseudomonas aeruginosa

P80: **Efficacy of tenofovir disoproxil fumarate treatment for Iranian chronic hepatitis B patients**

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Background and Aim: Tenofovir disoproxil fumarate (TDF), the oral prodrug of tenofovir is a new effective treatment option for patients with chronic hepatitis B that inhibits viral polymerase–reverse transcriptase. This nucleotide analog is structurally closely related to adefovir, but shows superior efficacy to adefovir dipivoxil in treatment of chronic hepatitis B. We evaluated efficacy of TDF monotherapy in patients with chronic hepatitis B who were positive or negative for hepatitis B e antigen (HBeAg- or HBeAg+).

Methods: A total of thirty patients (21 males and 9 females; median age 38 years, range 21-63 years) with chronic HBV infection were treated with TDF for at least 6 months. Case records were reviewed to obtain clinical and virological data. Efficacy end points included levels of HBV DNA, biochemical and serological response, and development of resistance mutations through treatment. The full-length of reverse transcriptase (RT) region from HBV DNA was amplified by nested polymerase chain reaction (Nested-PCR) from all samples and the PCR product was directly sequenced and compared with reference HBV data.

Results: At a median period of 41 week (range, 24-96), 67% of HBeAg- and 42% of HBeAg+ patients treated with TDF had levels of HBV DNA < 400 copies/mL. overall, 75% of HBeAg- and 67% of HBeAg+ patients had normalized levels of aminotransferases (ALT, AST) and none of them had HBeAg and HBsAg seroconversion. Amino acid substitutions in RT region from HBV DNA that are associated with resistance to TDF were not detected in any patient and phylogenetic analysis showed that all (100%) of patients were infected with HBV genotype D.

Conclusion: This study shows that TDF is effective in the management of Iranian HBeAg- and HBeAg+ patients with chronic hepatitis B. TDF monotherapy can control HBV viremia without development of resistance mutations, but further studies in a larger population and in longer period of time are needed.

Keywords: Tenofovir Disoproxil Fumarate, hepatitis B, Iranian patients

P81: **Epidemiological study on the prevalence rate of Cryptosporidiosis and other parasitic enteropathogens in patients with gastroenteritis in East Azarbaijan province , 2012**

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Background and Aim: Cryptosporidiosis is one of the parasitic diseases with acute or chronic gastroenteritis caused by unicellular Cryptosporidium. Infection is occurred mainly by contaminated water or food with oocysts which are excreted by human or animal sources. Self-limiting gastroenteritis is seen in immunocompetent individuals, but in immunocompromised patients it causes a sever disease with slow recovery. In this study, ecological conditions, superficial water, domestic and industrial animal husbandry Azarbaijan as a province to have potential ability for high prevalence of parasitic diseases.

Methods: This descriptive study was conducted between June 2012 to June 2013 in Bonab ,Maragheh and Ajabshir cities which are located in East Azarbaijan province. Overall, 600 stool samples of gastroenteritis patients were collected, fixed and examined by direct method (DM) for diagnosis of enteropathogenic parasites, acid-fast staining (AFS) and auramin phenol flourescence (APF) for detection of Cryptosporidium.

Results: The results confirmed the overall prevalence rate of parasitic infections was 2.6% among those cities, and the highest rate of infection was among Giardia lamblia 1.5%, Blastocystis hominis 0.8%, and Entamoeba coli 0.3% respectly. There was no infection with Cryptospordium in the test and control samples.

Conclusion: In conclusion, the current results showed a decrease in the rate of parasitic infections in Azarbaijan in compare with the previous studies, which indicated improvement in health education, water treatment, environmental sanitation and public knowledge.

Keywords:

P82: **Molecular detection of virulence factors ' pertussis toxin and adenylate cyclase toxin ' in Bordetella pertussis strains isolated from clinical samples in Iran**

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Background and Aim: Introduction: Whooping cough caused by Bordetella pertussis is a world-wide disease. B. pertussis is a strictly human pathogen with multiple biological activities and a gram-negative rod that was first isolated by Bordet and Gengou in 1906. The bacteria are transmitted by droplets and the infectious dose is small in immunologically naive patients. Pertussis toxin (PT) is a toxin secreted by the bacteria and composed of five different subunits. It is an A-B toxin. The B part is responsible for binding to the host cell and allows the A part to enter to the cell. The A part disrupts cellular functions via its ADP-ribosylating activity. Bordetella pertussis adenylate cyclase toxin (ACT), a hemolysin with enzymatic activity, is secreted in high concentration into the extracytoplasmatic space. In the present study, we amplified and sequenced ptx and cya genes to further studies about polymorphism of these virulence factors and comparison of the allels with standard strains.

Methods: Method: We examined 40 culture positive samples isolated from nasopharyngeal specimens collected in 2008- 2012. These strains have also been identified by biochemical tests. Regions of ptx and cya genes in these isolates of B. pertussis were amplified using specific primers by PCR method.

Results: Results: Our results showed that all examined strains in this study have ptx and cya genes in their genome in size 900 bp and 500 bp, respectively.

Conclusion: Discussion: Bsecause of polymorphism in these genes and its effect on bacterial virulence, especially in making vaccines against this disease, further studies to determine the predominant allele in isolates from patients and comparison with vaccine strains seem to be necessary.

Keywords: Bordetella pertussis- pertussis toxin-adenylate cyclase toxin

P83: **Survey on Association of the -59353T/C Polymorphism of CCR5 Gene with the Response to Interferon Treatment in Patients with Hepatitis C Infections**

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Background and Aim: CC-chemokine receptor (CCR) 5 is expressed on the several cells of the immune system and has been reported that the occurrence some of the polymorphisms in the promoter region of the encoding gene this receptor can be effective on susceptibility to infection or the response to antiviral treatment in hepatitis C virus (HCV)-infected patients. Hence, in this study the association between genotypes of CCR5-59353T/C polymorphism were examined with the response to interferon treatment in patients with hepatitis C infection.

Methods: Blood buffy coat samples were collected from 60 patients with HCV who were treated with interferon (30 sustained responders and 30 non-responsive patients) and 50 healthy controls. Genomic DNA was extracted by the salting out method. The genotypes of CCR5-59353T/C were identified using allele specific PCR method. The PCR products were electrophoresed on 1.5% agarose gel. Data were analysed using Chi-square test.

Results: The frequency of CCR5-59353CC mutant homozygous genotype in the patients who responded to treatment with interferon compared to non responders were 16% vs. 36%. There was no significant difference between them (P = 0.1). The frequency of C variant allele in responders to treatment compared to non responders were 45% vs. 58%. Also, there was no significant difference between the two groups (P = 0.2).

Conclusion: Based on the findings of this study, there was lack of association between genetic predisposition of this polymorphism with response to interferon therapy in HCV-infected patients. Therefore it is recommended due to various genetic and race in Iran, the next studies be performed on the more number of samples or the different races from other country regions.

Keywords: HCV, CCR5-59353, polymorphism, Interferon

P84: **Antibiotic resistance pattern and prevalence of TEM type beta-lactamase in clinical isolates of Pseudomonas aeruginosa in Mashhad, Iran**

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Background and Aim: Existence of extended spectrum beta-lactamase (ESBL) genes plays an important role in spreading of beta-lactam antibiotic resistance among bacteria. The resistance of Gram-negative bacteria, such as P. aeruginosa to different antimicrobial agents has increasingly been reported. This study was conducted to determine the antibiotic resistance pattern of P. aeruginosa clinical isolates in Mashhad and prevalence of TEM type beta-lactamase among them.

Methods: During the period of study in 2012, 60 clinical isolates of P. aeruginosa were collected from 5 hospital laboratories in Mashhad. Bacteria were isolated from different samples of hospitalized patients and identified by conventional biochemical tests. The antibiotic susceptibility was examined by disc diffusion method based on Kirby-Bauer standards. The frequency of ESBL producing strains was determined via the combined disk method according to Clinical Laboratory Standards Institute (CLSI). DNA was extracted using plasmid isolation kit (Sina Colon, Iran) and examined for the existence of blaTEM gene by polymerase chain reaction (PCR) using specific primers.

Results: Among 60 isolated bacteria, the highest resistance was to ceftizoxime (100%) and kanamycin (95.3%) and the lowest was to imipenem (45.3%). 54.7%, 9.4% and 35.9% of isolates were resistance, intermediate and susceptible to carbenicillin, respectively. Out of the 60 P. aeruginosa, 8(13.3%) were ESBL positive and none of them were positive for TEM beta-lactamase resistance gene.

Conclusion: Considering the increasing rate of the ESBL producing strains in our society, using the appropriate treatment protocol based on the antibiogram pattern of the strains is recommended. blaTEM gene was not found among isolated strains, so production of beta-lactamase is related to other types of ESBLs among isolated P. aeruginosa bacteria.

Keywords: Extended spectrum beta-lactamase, Pseudomonas aeruginosa, blaTEM gene, PCR

P85: **Comparative evaluation of IgG Avidity ELISA and IgM ELISA tests in differential diagnosis of acute T. gondii infection in early pregnancy**

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Background and Aim: Toxoplasmosis is a widespread zoonotic protozoon infection. The acute infection could cause drastic consequences in fetus of women infected in the first trimester of pregnancy. So diagnosis of acute infection in pregnant women especially in the first trimester is necessary. The aim of present the study was to evaluate the IgG Avidity ELISA and IgM ELISA tests in differentiation of acute and chronic infections of toxoplasmosis.

Methods: In a cross sectional study, 350 women in the first trimester of pregnancy admitted to the health care service centers of Hamadan city were chosen. After obtaining informed consent and demographic information from volunteers, their serum samples tested by IgG ELISA, IgM ELISA and IgG avidity ELISA methods.

Results: From total of 350 women,105(30%) were Toxoplasma IgG seropositive, of which 53 admitted again and after further sampling,¬ ¬two women¬(3.77%) showed rising IgG titers that one of them had miscarriage before the second sampling. IgG Avidity test confirmed the acute infection only in these two women and revealed the chronic infection in all the rest (51 women). But IgM ELISA¬¬ test, in addition to these two women, was weakly positive in one more woman with constant titers of IgG.

Conclusion: This study showed that the IgG Avidity ELISA test compared to the IgM ELISA¬ test is more suitable for discrimination of acute and chronic infections of toxoplasmosis and can be applied as a reliable diagnostic method in clinical diagnostic laboratories.

Keywords: Toxoplasmosis, Acute infection, IgG Avidity ELISA, IgM ELISA

P86: **Prevalance of L.monocytogenes in aborted featus and evaluation of its antibiotic resistance in Kazeroon sheep flocks**

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Background and Aim: Listeria monocytogenes is widely distributed in the environment and present the soil and the faeces of healthy animals. Bacterial pathogens were the most prevalent cause of abortion. Listeria monocytogenes is one of rare infective pathogens that could transferred via placenta during pregnancy and cause still birth or abortion in ruminants. Disaster of listeria is it could not produced and prevalent signs in pregnant animals. The most important cause of infective abortion of sheep and cow are infective and contagious and zoonosis between human and animals. That they are very important in public health .the aim of this study was to determine prevalence of Listeria monocytogenes in aborted featus of sheep in kazeron

Methods: The sheep fetus samples were collected from flocks in kazeron during autumn to winter of 2011 (n = 50). Then abomasumal content and tissues of aborted fetus of sheep were prepared by autopsy. Samples were subjected to microbiological and antibiotic screening test. Samples of abomasumal content and tissues enriched by cold enrichment method and cultured on blood agar and listeria selective agar and confirmed by biochemical test MRVP, catalase, hemolytic and cAMP activity.antibiotic screening tests were carried out on Muller- Hilton agar and antibiotic disc method. Results were evaluated with standard strain of PTCC1163.

Results: The contamination rate of L. monocytogenes was 36% (18), others bacteria 56% (28) and non contaminated 8% (4), respectively. All isolated Listerial colony were detected sensitive to ampicillin and tetracycline and resistant to penicillin and erythromycin. Standard control strain was shown same results

Conclusion: Our results were shown relative contamination to listeria monocytogemnes in aborted sheep fetus that could be a public health concern.

Keywords: Abortion; sheep ; Listeria monocytogenes;fetus

P87: **Isolation and identification Listeriosis factors, brucellosis and campylo bacteriosis Kazeroun city of sheep abortions evaluation of antibiotic resistance**

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Background and Aim: Bacterial diseases such Listeriosis, campylo bacteriosis and brucellosis has been published widely around the world and the importance of not only economic losses to livestock diseases are not confined to humans but important aspect of it has doubled. Reproductive abnormalities such as miscarriage and premature deliveries may be the only clinical signs of bacterial diseases in pregnant women and are ruminants like cattle and sheep.

Methods: ٥٠ samples of tissue from aborted fetal sheep abomasum abomasum and contents of each of the samples collected and cultured on two Bladagar environment and another one inside the jar in the refrigerator was incubated co٢. Suspicious colonies on Brucella-specific environments, and Listeria were cultured Campylobacter. All colonies for identification and final confirmation by Gram stain and biochemical tests and the Antibiogram were examined.

Results: 18 samples (36%) contaminated with Listeria, 12 samples (24%) contaminated with Brucella, 8 samples (16%) contaminated with Campylobacter, 4 samples (8%) without any contamination, and 8 samples (16%) infected with other infectious agents were. All isolates Listeria to tetracycline, Arytromaysn and SXT susceptible to ampicillin and penicillin Celine resistant isolates of Brucella than doxycycline, trimethoprim, sulfamethoxazole, SXT Vjntamaysyn sensitive and vancomycin-resistant and 3 isolated Campylobacter than cephalotin and 5 samples to Nalydyk acid-sensitive and all Campylobacter isolates relative to chloramphenicol and tetracycline sensitivity and were resistant to erythromycin.

Conclusion: Given the prevalence of in all three bacteria.And complications of bacterial infection.And since this study It was the first time in the region,Need refinement and evaluation of contamination in dairy products and meat samples are.

Keywords: abortion-sheep-Brucella-Listeria-Campylobacter

P88: **The comparison of Hemophilus influenzae carrier state in HIV infected children with healthy children under 6 years old of age**

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Background and Aim: Haemophilus influenzae (Hi) represents a group of bacteria that may cause different types of infections in infants and children. Pneumonia is one of the most important infections in this group especially in patients infected with human immunodeficiency virus (HIV). The mortality attributable to H. influenzae pneumonia is 11.5%. Immunocompromised persons can be affected more often than healthy children. The objective of this study was to determine the epidemiological prevalence of carrier in HIV positive children in comparison with a healthy one.

Methods: In this study, 60 samples had been collected since August 2011 to January 2012 at the Children Medical Center Hospital in Tehran. Thirty cases were HIV-infected children and 30 cases were healthy children as a control group. Nasopharyngeal swab specimens were collected from each child and then were placed in a modified Stuart transport medium and carried to the laboratory. The samples were cultured on chocolate agar plates supplemented with bacitracin. Primary identification for Hi was done by standard methods. PCR was used to confirm H. influenzae, based on omp6 gene.

Results: The results illustrated that 23 (76%) cases of the total 30 HIV infected children (16 males and 14 females) were carrier of H.influenzae ; 8 (35%), 15 (65%) cases of them were females and males respectively. Of 30 healthy children (15 males and 15 females) 9 (15%) cases were carrier of Hi; 4 (45%), 5 (55%) cases of them were females and males respectively. From 23 H.influenzae carrier of the HIV infected population and 9 children of the healthy population studied, 19 (83%) and 3 (33%) cases of them suffered from upper respiratory infections respectively. serotyping results showed that 25(83%) isolated from HIV infected children were nonetypeable serotype.

Conclusion: As expected, we have found that there is a strong relation between H. influenzae carrier and HIV infection. Twenty three (76%) cases of HIV infected children were positive for Hi, while among 30 healthy children only 9 (15%) were Hi-positive. These data have shown that HIV infected individuals are approximately 5 times more colonized by H.influenzae. Thus, infection caused by H.influenzae, effects mainly patients with advanced HIV disease. Furthermore our results suggest that since Hib did not colonize in these children, therefore most of them were infected by other nonetypeable serotype

Keywords: HIV-infected children, Haemophilus influenzae, PCR

P89: **High Prevalence of Panton—Valentine leukocidin gene among community acquired S.aureus isolated from Iranian healthy student**

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Background and Aim: Staphylococcus aureus nasal colonization is common among humans, and causality between carriage and invasive diseases has been substantiated. Panton—Valentine leukocidin (PVL), one of these pathogenic determinants, is a bicomponent cytotoxin encoded by the pvl genes luk-S-PV and luk-FPV. The PVL genes are predominantly associated with S. aureus strains that cause community acquired infections, including skin and soft-tissue abscesses, necrotizing pneumonia and invasive osteomyelitis. A cross sectional study was carried out to investigate the prevalence of pvl gene among S.aureus isolated from Iranian healthy student.

Methods:: A total of 568 nasal swabs were collected from healthy students at Arak university of Medical sciences located in center of Iran. All samples were subjected to S. aureus–specific isolation procedures (catalase, Coagulase, clumping factor, DNase, thermostable nuclease, 162 bp of sa442 gene), all strains were subjected to PVL toxin gene PCR.

Results: 84 S. aureus strains isolated from the anterior nares of 568 healthy students. Among the 84 community acquired Staphylococcus aureus, 37.6 %(32) isolates were PVL PCR positive which was confirmed by sequencing.

Conclusion: Our study shows that prevalence of PVL among carriage S. aureus(37.6%) isolates is higher than many previous reports. molecular epidemiology of PVL-positive strains is essential to monitor their spread in the community and its association with infections.

Keywords: community acquired Staphylococcus aureus, pvl, nasal carriage

P90: **Quantitative Real-time PCR assay targeting caf1gene of Yersinia pestis**

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Background and Aim: Yersinia pestis, the causative agent of the plague, is a major concern of public health in many countries as well as a potential bioweapon. Culture based methods to detect the organism are time consuming and troublesome. Thus Real-time PCR may provide a more specific and sensitive alternative for rapid detection of the organism. The aim of the study was development of a quantitative Real-time PCR assay based on caf1gene of Yersinia pestis.

Methods: The diagnostic primers and FAM/BHQ1dual labeled probe for the F1 capsule antigen gene (caf1) were designed using Allele ID 7.6 software. Setup reactions were performed in a standard volume contained Y.pestis genomic DNA as template. To construct an external positive control, the PCR product was cloned into pTZ57R/T plasmid vector. 10-fold serial dilutions of the pTZ-caf1 were subjected to the caf1 real-time PCR assay and the last dilution showing an acceptable signal was firmed as the limit of detection. Depicting a standard curve based on the Ct values, the assay was advanced as a quantitative tool. To determine the analytical specificity, the genome of some control negative bacteria was subjected to the assay.

Results: The amplification curve analysis confirmed specific detection of caf1 sequence without nonspecific signal. The last dilution of pTZ-caf1 plasmid that showed a fluorescent signal in FAM channel was 1 fg. So, the lower detectable copies number of the gene in a 20 µl PCR reaction was calculated as 3×102. The linearity for the quantitative assay was in the range of 1fg-10ng of pTZ-caf1 (3×102-3×109copies). The slope of -3.441, Y-intercept of 44.752, R2 of 0.1 and amplification efficiency of 95.252 were the specifications of the regression line for the standard curve generated by the caf1 TaqMan real time assay

Conclusion: This study is the first report of developing a Y. pestis quantitative real time PCR assay in IR Iran. Domestication and design of the assay is an important stride towards flourishing and development of medical diagnostic laboratories for exact diagnosis of the pathogens.

Keywords: Yersinia pestis, plague, diagnosis , Real-time PCR

P91: **HTLV-1/2 prevalence in Guilan blood donors of Iran**

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Background and Aim: Human T-cell lymphotropic virus (HTLV1)belongs to the family Retroviride and subfamily Enchovirine and it is first recognized human retrovirus . It is known pathogen of Adult T-cell leukemia / lymphoma (ATLL)2 , HTLV-1 Associated Myelopathy / tropical spastic paraparesis HAM/TSP3 HTLV infection transforms through 3 routes: From mother to the child especially during breast feeding , through sexual contact especially it is transferred by men to women and finally through transfusion of contaminated blood and its product or contaminated needles . According to the fact that khorasan province is one of endomic regions for this virus and on the other hand it's outbreak in other provinces is possible , we tried to study its occurrence rate amongst blood donors in Eastern Guilan region .

Methods: In this descriptive-analytic study , 25348 serum of blood donors including 24355 males and 993 females were investigated .Their serum were Anti HTLV screened by using ELISA. positive cases in primary screening tests were tested again in two fold method (pilot and cord of blood bag) under ELISA test . Positive case were validated by western blot.

Results: According to the fact that the number of female donors were greatly lower than males, Anti-HTLV occurrence with 47 positive cases was 0.18 % , in primary test , it was 0.098% in secondary test with 25 positive cases and 0.043 % in validation test with 11 positive cases , where all of them were males .

Conclusion: Awareness rate of studied cases about this virus and its transformation method was very low and approximately 0.41 % .In present study , relation between age and infection rate was proved so that HTLV frequency rate will increase with age .On the other hand , relation between contaminated blood and its products injection and infection rate was observed. Among 11 positive cases of validation test , 5 cases had a blood injection history in previous years . Thus , general awareness , donated bloods screening and testing infected people especially infected mothers in endemic regions are among preventive method against propagation of this virus .

Keywords: Retrovirus , HTLV , serology , blood donors , Eastern Guilan

P92: **Antimicrobial effect of Origanum vulgare on Staphylococcus aureus in a rat model of**

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Background and Aim: The wound infection is one of the frequent complications in patients undergoing surgical operations. Staphylococcus aureus is the most common cause of surgical wound infections. Origanum vulgare has been shown to bear strong antimicrobial activity, especially against Gram-positive pathogens. This study was designed to investigate the antimicrobial effects of Origanum vulgare and the inhibition of growth of S. aureus in surgical wounds in a rat model.

Methods: Twenty male Sprague Dawley rats were divided randomly into two equal groups of treated and control rats. A circular incision was created on the dorsal inter-scapular region of each rat. After skin wounding, rats were inoculated locally with 1 × 104 CFU of S. aureus at sites of skin wounds. The extract was applied topically twice a day throughout the experiment. Animals of the control group were left untreated.

Results: The bacterial number in untreated animals was 7 × 106 ± 6 CFU/wound. These values were significantly different from those obtained from animals treated with extract of O. vulgare (2 × 105 ± 1 CFU/wound).

Conclusion: Results have revealed that topical application of O. vulgare extract on the infected wound sites produced significant antibacterial activity against S. aureus.

Keywords: Origanum vulgare, Surgical wounds, S. aureus

P93: **Molecular diagnosis of brucellosis-causing bacteria isolated from patients with brucellosis with the help of trpE and omp25 genes by PCR**

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Background and Aim: Brucellosis is a zoonotic disease in humans and animals. Every year, more than 500 thousand people are afflicted by it worldwide. Since, this disease is common in various parts of Iran and due to the losses caused by its spread, controlling and preventing brucellosis is viewed as a high-priority action. Nowadays, several serological tests for brucellosis diagnosis are available but all of these tests have their own limits. The aim with the present study is to use PCR according to omp25 and trpE genes for the molecular analysis of the individuals with brucellosis.

Methods: In this study, 30 clinical isolates were studied. All the patients were diagnosed with positive immunological tests such as Wright and Coombs. Samples of blood were cultured (BACTEC) and incubated at 37 degrees Celsius for 5 days and then they were cultured for 3 days on Brucella agar. DNA was extracted from the colonies by Kit. The extracted DNA was used as Template for accurate diagnosis of bacterial strains with gene-specific primers in PCR reactions omp25 and trpE.

Results: In this study all the samples selected were seropositive of from which the colonies were obtained. DNA extracted from the colonies by the use of kit was proved by spectrophotometer device. Also the results of the amplification reaction and the amplified bands of 486 and 490 bp for omp25 and trpE genes indicate the validity of the primers. By reproduction of these components, the genus Brucella was specifically identified.

Conclusion: Using the PCR technique, on the contrary to the serological diagnostic methods facilitates the tracing and identifying the bacteria especially those with slow growth rate. In this research, the trpE gene which has been not used in the diagnosis of brucellosis in Iran was exploited. The results of this study indicate that by amplification of some specific regions of the omp25 and trpE genes which are belonged to the conserved parts of the genus Brucella, identifying bacteria Brucella under PCR method is possible.

Keywords: brucellosis, PCR, BACTEC, immunological, protected

P94: **Prevalence of enterotoxin a, b genes in Staphylococcus aureus isolated from clinical samples and healthy carriers in Gorgan city, north of Iran**

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2- Infectious Disease Research Center, Gorgan, Iran
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Background and Aim: Introduction: Staphylococcus aureus (S. aureus) , a nosocomial and community acquired pathogen, is a major public health problem . wide infections caused by S.aureus from skin infections to systemic diseases are because of having several viru¬lence factors such as enzymes, toxins and also Enterotoxins. Enterotoxin A (SEA) , enterotoxin B(SEB) are superantigen and gasterointestinal toxins causing food poisoning . Sea, seb genes encode SEA and SEB , respectively. The goal of this study was determine the prevalence of sea, seb genes in S.aureus isolated from patients and healthy carriers in Gorgan city, north of Iran.

Methods: Methods: 170 isolates of S. aureus, 95 from patients and 75 healthy carriers, were collected during one year. After identification and purification, DNA extraction was done by phenol-chloroform method. Amplification of sea and seb genes was done by specific primers and polymerase chain reaction method.

Results: Results: Among the 170 isolates of S.aureus, 60.6% and 27.1% contained sea and seb genes, respectively. The frequency of isolates contained sea and seb genes in MRSA and MSSA isolates were 58.8%, 61.3% and 23.5% ,28.6%, respectively, which was not statistically significant. The frequency of these genes was not related to age, sex and source of isolation from patients.

Conclusion: Conclusion: This study showed high proportion of S. aureus isolates carry sea gene , whereas, The frequency of seb gene in this region was predictable.

Keywords: Keywords: Staphylococcus aureus; enterotoxin A; enterotoxin B;PCR

P95: **The bacteria causing urinary tract infection and their antibiotic resistance pattern in shahidmadani hospital ,Tabriz, Iran**

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Background and Aim: Background: urinary tract infection is one of the most common bacterial infection. Bacterial resistance to antibiotics is one of the failure treatment .So knowing common bacteria in every area and their antibiotic resistance pattern can help to choose proper antibiotic . the aim of this study was to determine the most common bacteria causing UTI in patients of Tabriz shahidmadani hospital at 1389 & 1390.

Methods: Materials & methods: this Descriptive - section study carried out on specimens collected form patients of women surgery & women internal of Tabriz shahidmadani heart hospital during a 2 year period . these 821 specimens cultured on blood agar & EMB plates and incubated in 37˚C for 24h .then antibacterial susceptibility test was performed by Kirby-bauer method for positive cases and the data were analyzed by SPSS software.

Results: Results: in this study among 821 urine samples , 121 samples had posetive culture. Frequency of various bacteria causing UTI was (75.39%)95 cases for E.coli , (9.52%)12 cases for pseudomonas , (3.96%)5 cases for Klebsiella.pneumoniae and some other bacteria. In this positive cases antibiogram determined that high degree of antibiotic resistance was shown for Erythromycin (83.34%), nalidixic acid (69.45%), co-trimoxazole(57.7%), cefexime(52.71%) and high degree of antibiotic sensitivity is shown for Imipenem(98.71%), Amikacin(95.74%), Meropenem(90%), ceftizoxim(88.88%) .

Conclusion: Conclusion: in our study showed that most common bacteria that causes UTI is E.coli , but some other bacteria like Klebsiella , Pseudomonas, Proteus and some others can causes this .and the most antibiotic resistance showed about Erithromycine. Specifically for E.coli as the most common bacteria causing UTI , high degree of antiiotic resistant was for ceftazidim and nalidixic acid and the lowest antibiotic resistance was for Amikacin and Meropenem.

Keywords: urinary infection , antibiotic , antibiotic resistance pattern

P96: **Molecular Diagnostics infectious causes of acute idiopathic mycocarditis and cardymyopathy in people with heart transplant by Method PCR**

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Background and Aim: Myocarditis is an inflammatory disease of myocardiom .Mostly they are not diagnose Because in the primary phases there is no particular sympthom.In case of not diagnosing and immediate therapy the disease will develop as cardiomyopatyand permanent enlargment of left venticular and lack of venticular contraction and in this phase heart transplantation is only curative therapy. The aim of this study was to investigate infectious causes(baceria) cardiomyopathy in patients with heart failure and heart transplant recipients in hospitals BaghiatollahAzam (AS) and MasihDaneshvari &shariati in molecular method (PCR)

Methods: About 15 samples of endomycards from 15 young people who suffered from heart failure with cardomypati who were repentance of heart transplantation in which with molecular method(PCR)higher prevalance of risk factors such as bacteria, staphylococcus aureus, streptoccuspyogenes

Results: According to the studies in developed countries and our study it seems that infectious factors and viruses are most prevalent causes of cardiomyopaty because of the significance of virus factors in causing cardiomyopaty diagnosing these factors immediately in primary phases and with sensetive and meticules molecular methods like PCR is necessary till with diagnosing infectious factors and determining frequency by presenting a method Anti-bacterial therapy stop the process of myocardit and intense heart failure and can be effective in reducing costs of heart failure and transplant

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Keywords: Myocardit,cardimyopaty, PCR.Hearttransplantation, Bacteria

P97: **The survey of bacterial infection in Burn patients**

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Background and Aim: Burn wounds are suitable environments for the growth of various opportunistic infections. The knowledge of the common microorganisms in these infections and their antibiotic resistance are fundamental. We studied common microorganisms and their antibiotic resistance in burn ward of Nekuei Hospital, Qom, Iran

Methods: In this study, during 5 months, 70 patients admitted to the burn ward of Nekuei hospital were examined. After Sampling and isolation of bacteria, biochemical standard tests for determination of microorganisms were done. Determination of antibiotic resistance was done by using disc diffusion or Kirby Bauer using these antibiotics: co-trimoxazole, vancomycin, ciprofloxacin, cephalothin, ceftazidime, amoxicillin, amikacin, gentamicin, chloramphenicol, cefazolin, cefotaxime, ceftriaxone, ampicillin, oxacillin, and imipenem

Results: Totally, the cultures of 54 cases (77.14%) of a total of 70 samples were positive. The most common isolated bacteria were Pseudomonas aeruginosa (38.9%), Staphylococcus aureus, and staphylococcus epidermidis (11.42%), and Enterococcus faecalis (9.59%). The results of the Antibiotic resistance of Pseudomonas aeruginosa are as follows: amoxicillin 94.73%, amikacin 25.64%, gentamicin 30.77%, co-trimoxazole 84.62%, ciprofloxacin 48.72%, ceftazidime 51.28%, cefotaxime 58.97%, Chloramphenicol 86.84%, ceftriaxone 55.26%, and imipenem 50%

Conclusion: The most common bacteria in infection of burn wound was pseudomonas aeruginosa, which was mostly susceptible to amikacin and gentamicin.

Keywords:: infection, burning , micro organisms

P98: **The study of the presence of the Pilus Islet-1 gene in Streptococcus pneumoniae isolated from clinical samples**

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Background and Aim: The study of the presence of the Pilus Islet-1 gene in Streptococcus pneumoniae isolated from clinical samples. Streptococcus pneumoniae is a major cause of morbidity and mortality worldwide. It is a main cause of upper respiratory tract infections a major cause of invasive disease in children under 2 years and the elderly people upper 65 years. The initial event in invasive pneumococcal disease is the attachment of encapsulated pneumococci to epithelial cells in the upper respiratory tract. In recent studies were shown the presence of the pili with the other organs enhance the ability of initial bacterial adhesion to epithelial cells. Two types (Pilus Islet-1 and Pilus Islet -2) have identified the presence of a pilus-like structure in S. pneumoniae . Plius Islet -1 are encoded by the pneumococcal rlrA islet. This locus consists of seven genes of which rrgA, rrgB, and rrgC also contains genes for three sortases, srtB, srtC, and srtD, as well as rlrA (a positive regulator of the gene cluster).

Methods: A total of 90 isolates were collected from clinical specimens. The isolation sites were from 10 CSF , 19 blood , 7 keratitis, 6 trachea, 14 sputum, 7 cornea, 9 conjectivitis, 3 secretions of ear, 3 plural effusion, 6 sinusitis , 1 acite fluid , 1 Bal , 1 otitis media, 3 urine. DNA was extracted using DNA purification kit and PCR performed with rlrA gene as a marker for the pilus islet-1.

Results: Overall, 39 (43.33%) of 90 isolates were positive for the rlrA gene. The positive isolates were from: 2 (5.1%) CSF, 9(23%) blood culture, 3(7.6%) keratitis, 3(7.6%) trachea, 5(12.82%) sputum, 5 (12.85%)cornea, 5(12.85%) eye, 3(7.6%) sinusit, 1(2.4%) asit ,1(2.4%) Bal, 1(2.4%) conjectivit eye , 1(2.4%) urine.

Conclusion: These results show high prevalence of the pilus islet-1 in pneumococcal isolates. Furthermore the prevalence of this gene in invasive isolates, blood and CSF were more than other isolates. The high pilus prevalence is indicating the potential of isolates for more attachment in invasive isolates.

Keywords: pili, prevalence, clinical isolate

P99: **Identification and Prevalence of Klebsiella oxytoca in antibiotic-associated colitis and Investigation of bla-CTX-M ,bla-TEM, bla-SHV and bla-OXA genes in ESBLs types**

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Background and Aim: A type of colitis with bacterial origin is antibiotic associated colitis.This type of colitis is due to toxin-producing Clostridium difficile.The other type of colitis which is the main complication, is antibiotic-associated hemorrhagic colitis would be seen after antibiotic therapy by Beta-lactams, Cephalosporins and Quinolones.The last surveys show that one of the causes of colitis is the species of Klebsiella oxytoca. The aim of this study is to identify Klebsiella oxytoca in antibiotic-associated colitis and to investigate the antibiotic resistance pattern.

Methods: In this study,fecal samples were collected from hospitalized patients who had diarrhea after use of antibiotic. The samples were transferred to supportive media. Primary culture and differentiation on the specific media were utilized to diagnose K.oxytoca. Eventually, bacterial samples were confirmed by PCR method for detection of a specific gene in K.oxytoca (polygalacturonase pehX gene).The pattern of antibiotic resistance were investigated by using Kirby-Bauer disk diffusion method. The type of ESBLs produced by these strains was determined by using PCR method and also by using ESBLs phenotypic confirmatory method.

Results: The number of 331 samples were collected from patients who had diarrhea 1 week to 2 months after starting antibiotic therapy. After analysis by microbial and biochemical tests,57 strains of K.oxytoca were identified.Totally 40(12.8%) strains of K.oxytoca were confirmed for specific gene by PCR technique. The pattern of antibiotic resistance analysis showed,that 40 isolates were susceptible to Amikacin 97%, Gentamicin 87%, Imipenem 92% and Meropenem 90%, Ampicillin/Sulbactam 67%,Cotrimoxazole 67% and the group with Cephalosporins 72%.These results were confirmed by standard method recently published by CLSI criteria. There are 12 isolations of ESBLs-producing strains. The PCR showed that 20% blaCTX-M , 98% blaTEM , 18% blaSHV and 10% blaOXA nucleotide identification.

Conclusion: Following the investigations in other countries, our results confirmed, there would be a particular attention to Klebsiella oxytoca among other pathogens causing colitis.

Keywords: Hospitalized patients , antibiotic-associated colitis , Klebsiella oxytoca , antibiotic-resistance, ESBLs

P100: **Sequence analysis of N gene in measles virus AIK-C strain used for vaccine production at Razi institute**

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Background and Aim: The AIK-C strain of measles virus is used to produce vaccine at Razi vaccine and serum research Institute. Previously, the mentioned virus was grown on fibroblast cells, which was later, adapted to MRC-5 cells. The c-terminal region of N gene in this virus is highly variable and may mutate after change in cell line. Any chnage in the genes may thus effect the safet and potency of the vaccine. Hence, this study was undertaken to monitor possible changes in the mentioned gene after adaptation on MRC5 cells.

Methods: The RNA virus was isolated from MRC-5 cell and cDNA of the nucleocapsid gene was created and cloned by two method of direct sequencing. After cloning, the mentioned gene was sequenced using a pair of universal primers and an internal primer N1 278-295F. The obtained nucleotide sequence was compared with the sequence of the viral strain grown on fibroblast cells (submitted previously in gene bank).

Results: The N gene of measles virus AIK-C is approximately 1580 bp. During PCR analysis a bond of 1600bp was achieved which was sequenced using the internal primer. According to results, no change in the sequence of the strain used for vaccinal production and the previously submitted strain was observed

Conclusion: Based on previous reports and the results of this study, it is suggested to use this clone of nucleocapsid of measles virus as a standard for quantitative real time PCR instead of CCID50 method.

Keywords: Measles virus, MRC-5 cells, Vaccine, Nucleotide sequencing

P101: **CASPASE 3/7 ACTIVITIES MEASUREMENT OVER A RANGE OF CyaA TOXINS CONCENTRATIONS ON DIFFERENT CELLS.**

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Background and Aim:: Adenylate cyclase toxin (CyaA) toxin is an important virulence factor of Bordetella pertussis, the causative agent of whooping cough, and a potential component of acellular pertussis vaccine.

Methods: The work involved the production of three purified forms of CyaA with different enzymic and invasive properties. These were: the native enzymatically-active, invasive toxin (CyaA), an invasive derivative lacking AC enzymic activity (CyaA*) and a non-acylated, non-invasive form of CyaA (proCyaA). These were expressed in E. coli BL21/DE3 as recombinant proteins. After purification by a combination of chromatographic methods (Q-and Butyl-Sepharose) their properties were investigated by several assays.

Results: The AC enzymic activity was assayed by a conductimetric method. CyaA and pro-CyaA had a high level of enzymic activity but that of CyaA* was very low. Caspase 3/7 activities were measured over a range of toxin concentrations. At these concentrations, neither urea buffer alone nor CyaA* induced any significant increase in caspase 3/7 from different mammalian cells. The greatest effect of CyaA was observed on J774.2 and RBL-2H3 cells where increasing concentration of toxin gave increasing activity.

Conclusion:: regard to the results of this the study showed that both enzymatic and invasive functions are required for the cytotoxic effects of adenylate cyclase toxin.

Keywords: Adenylate cyclase toxin ,Caspase 3/7,Bordetella pertussis

P102: **Enhanced Erythromycin Production Using Magnetic Nanoparticles in Saccharapolyspora erythraea Growth Culture**

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3- Nanotechnology research center, research institute of petroleum industry, Tehran, Iran.

Background and Aim: Evidence shows that use of nanoparticles in the growth culture of microorganisms has the potential to increase production of secondary metabolites. In this study, we examined the impact of using magnetic nanoparticles on microbial production of erythromycin by Saccharopolyspora erythraea.

Methods: Magnetic nanoparticles Fe3O4 and Fe2O3 were synthetized using co-precipitation method. Size of nanoparticles was determined to be 30 nm. The fermentation media was supplemented with different concentrations of nanoparticles, including 0, 5, 2, 8 %w/w. The effect of nanoparticles presence on microbial growth, microbial morphology, biomass, and Erythromycin concentration was investigated during a 10-day fermentation process.

Results: Our results indicated a significant higher Erythromycin concentration as well as biomass production in the nanoparticle supplemented medium as compared with nanoparticle free medium. The optimized concentration of nanoparticles was identified to be 2% w/w, which leads to a 2.5 time higher fermentation yield in comparison with nanoparticle free fermentation process.

Conclusion: Our results indicate that presence of magnetic nanoparticles in S. erythraea growth culture can lead to a higher Erythromycin production yield. This study provides a baseline for further large-scale studies exploring the impact of magnetic nanoparticle use for enhanced industrial production of Erythromycin.

Keywords: fermentation, Erythromycin, Saccharopolyspora erythraea, microbial growth,magnetic nanoparticles

P103: **Evaluation effects of Aged garlic extract (AGE)and aflatoxin-B1(AFB-1) on Matrix methaloporotein 3 and 9 on serum of normal and toumoric Balb/C mouse**

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Background and Aim: Background: AFB-1is one of the most important mycotoxins that is produced by Aspergillus flavus on foods including cereals, milk, nuts and metabolized in the body and creat toumur. AGE is indigenous medicinal plants in Iranian traditional medicine that is effective in the treatment of cancer has attracted much attention. This study examined the effects of immunomedulatory garlic and aflatoxin-B1 in normal and breast cancer mouse models.So it has been recommended method for early detection of tumor invasion.

Methods: Materials and Methods: In this experimental research, AFB1 was separated from As¬pergillus flavus (PTCC 5004) by HPLC and AGE prepared using the Mantis method. The Delayed-Type Hypersensitivity (DTH) test was carried out to determinate the effectiveness of different doses of AGE and AFB1, which can both have an effect on the immune sys¬tem. Subsequent experiments were carried out on 40 Balb/c mice to estimate the effects of AGE and AFB1 on the percentage of mmp3 and mmp9 in 4 groups: 10 µl/kg/day of AFB1 and AGE diluents were administered for 4 consecutive days to group 1: AFB1, 2: control; 3: AGE + AFB 1 and 4: AGE via intraperitoneal (IP) route, respectively. Blood samples were taken of Corner of eyes of mice and Separated Serum of boold and measured the percentage of mmp3 and mmp9 by ELISA method.

Results: Results: The results showed that AGE lowered mmp 3 and 9 cocentration in blood serum of mouse and aflatoxin-B1 raised it. ((P-value <0.05)

Conclusion: Conclusion: AFB-1, is one of the most important risk factors for cancer.This disease has multiple causes that require treatment with multiple objectives. AFB-1supress immune system and causing aggressive Toumor and the worse prognosis by increase mmp 3 and 9 cocentration in blood. Aged garlic extract increased immune system and improvement of toumor. Thus eliminating AFB-1strategy of daily food and consumption of AGE the appropriate choise in the treatment of cancer, to decline mortality of cancer ,should be considered more serious. So, AGE might be used as herbal medicine with few side effects as compared to chemotherapy in treating cancers caused by substances like AFB1.So early detection of cancer,in Early stages by determination of persentage of mmp 3 and mmp 9 in the serum of individuals suspected of advances cancer can be prevented.

Keywords: Keywords: Aflatoxin-B1, cancer, Garlic, Matrix methaloporotein 3 and 9.

P104: **Contamination of recreational water sources to Acanthamoeba spp. in Tehran, Iran**

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Background and Aim: Acanthamoeba spp. is the ubiquitous potentially pathogenic free-living amoebae (FLA) in nature such as soil, clays and various water sources such as fresh water, sea water and man-made water sources. Recently Acanthamoeba keratitis (AK) continues to rise in Iran. Interestingly, most of patients report a history of contact with water sources before the onset of disease. The main aim of the present study was to determine the occurrence of Acanthamoeba spp. in the recreational water sources of Tehran, Iran using morphological and molecular based tests

Methods: Overall, 50 samples were collected from recreational water sources including man- made and natural waters in Tehran province. Filtration and cultivation of samples were done using non-nutrient agar overlaid with heat inactivated Escherichia coli. Cloning of Acanthamoeba spp. was then performed to eliminate bacterial and fungi contamination. DNA extraction were done using phenol chloroform procedure and Polymerase Chain Reaction (PCR) were performed using genus specific primer pairs called JDP1-2 (genus-specific primer pair).

Results: Out of 50 water samples, 11 were positive for Acanthamoeba trophozoites and cysts according to morphological criteria. Double walled cysts and flat trophozoites moving slowly in the plates were the main character of amoebae diagnosis. Cloning of all isolates was done successfully. All of isolates present a 500 base pair PCR band which is specific to Acanthamoeba genus. The positive water sources were mainly used for washing purposes.

Conclusion: Presence of Acanthamoeba in recreational water sources is of concern for high risk people. The present study is the second to identify Acanthamoeba in recreational water sources. posting of alarming sign and education to high risk people is of utmost importance to prevent Acanthamoeba related infections.

Keywords: Acanthamoeba, Recreational water sources, PCR, Morphology

P105: **Isolation and Molecular identification of AspergillusppIsolated from ICU, indoor and outdoor environments hospitals in Mazandaran Province and determine Antifungal susceptibility**

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Background and Aim: Aspergillusspp is able to germinate and grow over a wide range of temperatures and pH conditions, increasing its ability to adapt to adverse environments. While exposure to Aspergillus spores is universal, many causal arguments for hospital acquisition of IA can be put forward

Methods: From May 2012 through October,500 plates of SDA was placed at a height of 1m above the ground in inside and outside of the ICU of Mazandaran hospitals, then identify the approximate according to macroscopic and microscopic morphology. The broth microdilution assay was performed according to the Clinical and Laboratory Standards Institute NLCCLS– M38A method. DNA sequencing of the ITS1 and ITS4 regions and was also differentiation of species conducted by method Melting Real-Time PCR. Species identification was based on sequence similarity using BLAST searches. Sequences were compared to those derived from type strains deposited in the Gen Bank data base to identify isolates to the species level

Results: 350Apergillus strains were obtained from ICU, indoor and outdoor environments hospitals in Mazandaran Province. 15% of the colonies were Aspergillus fumigatus,35% Aspergillus niger and 50% were Aspergillus flavus colonies .In this study whith molecular method were 100% hemology between the Aspergillus isolates of indoor and outdoor the ICU, also were recorded some isolates as a new strains in Gene Bank. The MIC of Isolates A.flavus and A.niger, were According to refrence strains of NLCCLS– M38A method and internal studies, but demonstrated some of isolates Aspergillusfumigatus with MIC?4, less sensitive compared to references strains of NLCCLS– M38A method and internal studies.

Conclusion: The Melting Real-Time PCR is a rapid, accurate and suitable method for identification of Aspergillus. Also according to findings, the increase of MIC shows high resistance of Aspergillus fumigatus to Antifungal itraconazole in Iran and mazandaran.

Keywords: Melting Real-Time PCR ,Aspergillus,Icu,Antifungal susceptibility,Itraconazol,ITS

P106: **Antimicrobial susceptibility trends among Streptococcus pneumoniae over an 11-year period in an Iranian referral children Hospital**

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Background and Aim: The appearance of antibiotic resistance in Streptococcus pneumoniae has raised a global concern over the past three decades. This study was conducted to determine the antimicrobial susceptibility of S. pneumoniae isolated from patients in Children´s Medical Center Hospital during 2001 to 2011.

Methods: During the 11 years period, a total of 194 S. pneumoniae isolates were collected and time series analysis of the most prevalent antibiotics was performed.

Results: Time series analysis of the 5 antibiotics of penicillin, erythromycin, chloramphenicol, ceftriaxone, and trimethoprim-sulfamethoxazole showed an overall decreasing trend for S. pneumoniae susceptibility during 2001 to 2011 and even forecasting prediction for 2016. The prevalence of susceptibility to penicillin decreased from 78% in 2001 to 32% in 2011. In the same time period, susceptibility to erythromycin declined from 75% (in 2001 to 35% in 2011 and susceptibility to chloramphenicol started to decrease from 94% to 55%. In addition, during these couple of years, susceptibility to ampicillin declined from 70% to 62%. Beside this, susceptibility to ceftriaxone started to fall from 100% to 87% and susceptibility to sulfametoxazole went down from 57% to 40%.

Conclusion: This study identifies unstable patterns of resistance to available antimicrobial drugs during 11 years. Continued epidemiological surveillance appears to be prudent practice to guide effective chemotherapy. Moreover, it would be an important key to consider antimicrobial stewardship as an essential factor to prevent the development of antimicrobial resistance.

Keywords: S. pneumonia, antibiotic resistance, Iran

P107: **Clonal spread of vancomycin resistance Enterococcus faecalis in an Iranian referral pediatrics center**

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Background and Aim: Vancomycin-resistant enterococci (VRE) represent as an immediate threat to public health. Since few active compounds are available for VRE infections, rapid identification of these isolates are essential. In the absence of any report on the genetic relatedness of Enterococcus faecalis especially Vancomycin-resistant E. faecalis (VREF) isolates in Iran, we undertook this study to characterize these isolates using random amplification of polymorphic DNA (RAPD–PCR) genotyping method.

Methods: In this study, E. faecalis strains isolated from various samples collected from different wards of Children Medical Hospital (Tehran, Iran). These isolates were identified by standard laboratory procedures and tested for antimicrobial resistance to vancomycin and teicoplanin. The genetic similarity of the strains was investigated by amplification of the RAPD–PCR.

Results: In our study among 91 E. faecalis isolates, 15 (16%) were identified as VREF. The similarity pattern built for E. faecalis isolates by RAPD–PCR, demonstrated the presence of four distinct clusters (A, B, C, D). It is of interest to note that 100% of VREF isolates belonged to Clusters A, indicating that there may have occurred horizontal transmission of the same strain between patients.

Conclusion: In conclusion, rapid spread of VREF from a clonal origin calls for implementation of careful isolation and infection control measures. Therefore, environmental control by routine disinfection of patient area as well as screening of high risk patients and isolation of colonized patients should be imposed in order to diminish risk of acquiring nosocomial VRE.

Keywords: Enterococcus faecalis, Genotyping, Vancomycin-resistant

P108: **Study of molecular epidemiology of bovine rotaviruses in diarrheic calves in province of Tehran by real time-PCR method**

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Background and Aim: Group A rotavirus (RVA) is the most common cause of severe gastroenteritis associated with significant morbidity, mortality, and economic burden among children and newborn calves worldwide. Classification of rotaviruses group A is on the basis of two external neutralizing antigens capsid proteins VP7 and VP4 which established dual genotyping system defined as G (glycoprotein) and P (protease sensitive protein) genotypes, respectively. Nowadays, RT-PCR coupled with multiple semi-nested PCR are using to determination of G and P genotypes. Using semi-nested PCR has some disadvantages such as low efficiency and contamination problem. Furthermore these sequentially PCRs are time-consuming. Aim of this study was establishing a multiplex real-time polymerase chain reaction protocol using primers and cyber green dye for evaluation of prevalent genotypes of VP7 genes. This assay offers simultaneous genotyping and quantification of the most common RVA G genotypes G6, G8 and G10.

Methods: Rotavirus genomic RNA was extracted from 50 bovine rotavirus positive stool samples and also from MA104 cells. Specific primers for real time PCR were designed for detection of G6, G8 and G10 genotypes of rotavirus with considering of presence of a difference in products Tm. The primers were designed by Allele ID v6.0 and Beacon designer.v7.01 softwares. Before running the real time system, amplification of the related products was checked by conventional PCRs. Then, real time PCR was performed by 7500 Real Time PCR System.Finally, results were analyzed by 7500 System Software.

Results: Genotyping analysis among the G genotyped samples indicated that G6 was the most prevalent G genotypes (about 50%) followed by G10 (about 37.5%) and then G8 (about 8 %), and, the rest were a mixed genotyped of G6 and G10. To examine the reliability of the established methodology, the common published primers for the conventional nested PCR assay for detecting and genotyping bovine rotavirus group A were used. The results revealed complete agreement between the conventional nested PCR assay and the proposed real time multiplex PCR.

Conclusion: To our knowledge this is the first study where a RT-real time PCR assay can be used for the concurrent detection, quantitation and genotyping of the most prevalent types of bovine rotaviral genome. Also, without doing an expensive test, it quickly provides valuable information offering a reliable and useful tool for the necessary prospective molecular epidemiological studies.Since in this new methodology, the size of the PCR products is samller than the conventional PCRs, the sensitivity of the assay enhances, too.

Keywords: Rotavirus, Bovine, Genotyping, Realtime PCR

P109: **Evaluation of S.griseofuscus antagonistic activity against isolated bacteria from infected urethral tracts**

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Background and Aim: The Streptomyces sp are the most valuable prokaryotic cells in economical and biotechnological manner. They produce about half of bioactive secondary metabolites including antibiotics ,antitumor agents and enzymes.

Methods: The six bacterial species ,Acinetobacter sp and identified E.coli ,K.pneumoniae ,S.marcescens, P.aeroginosa and P.mirabilis were isolated from urine patients according to differential and selective tests. It was used spot inoculums and pour plate procedures for assessing of antagonistic activity of S. griseofuscus strain against clinical isolate. The submerge method was used for production of antimicrobial materials using ISP2 medium. Finally ,The agar well diffusion method was used for final screening for antibiotic susceptibility.

Results: S.griseofuscus Indicated had antimicrobial activity against E.coli and S.marcescens using spot inoculums. Also, it had antimicrobial activity against on E.coli and Acinetobacter using pour plate method. This activity had been showed on S.marcescens, P.aeroginosa P.mirabilis, Acinetobacter sp, E.coli and K.pneumoniae using agar well diffusion method and ISP2 medium was used for Producing Bioactive Substances. produced bioactive components by S. griseofuscus were identified using GC/MS. The separated components of bioactive compounds including 3-hexene-2-one ،1,1-Diethoxyethane (Diethyl acetal) ،1-Propoxy-2-propanol ،2,6-dimethyl-2,5Heptadien-4-one ،2-Heptanol ،Eicosane and 10-methyl elcosane.

Conclusion: The demonstrated antibacterial effect of This strain suggests that it may be one of new medicinal resources for antimicrobial agents.

Keywords: Streptomyces, Antimicrobial activity, Agar well diffusion method

P110: **Constitutive and inducible clindamycin resistance incidence in Staphylococcus aureus isolated from clinical samples**

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Background and Aim: S. aureus infections were historically treatable with common antibiotics; emergence of drug-resistant organisms is now a major concern. Antibiotic resistance to macrolide family in Staphylococcus aureus may be due to activation of efflux pumps that leads to resistance to macrolides and type B streptogramins (MSB) or may result from a modification in the ribosomal target, as a leading cause of resistance to macrolides, lincosamides and type B streptogramins(MLSB). MLS antibiotics are commonly used for the treatment of staphylococcal infections, clindamycin are a common choice for some of staphylococcal infections. Methylases confer inducible (Inducible MLSB or MLSBi) resistance cannot be identified by standard methods of antibiotic susceptibility testing. Failure in detection of MLSBi resistance may result in clinical failure to clindamycin treatment. (D-test) appears to be a reliable indicator of MLSBi strains.

Methods: This cross-sectional study was performed on a total of 200 S. aureus isolates from 302 S.aureus which were collected from two teaching hospitals in Shiraz (Namazi and Faghihi hospitals) during 2012. The isolates were defined as S. aureus at the Microbiology Laboratory using standard procedures. Methicillin resistant S. aureus were screened based on their resistance to 30µg cefoxitin disks. 100 MSSA (methicillin-sensitive Staphylococcus aureus) and 100 MRSA (meticillin resistance Staphylococcus aureus) isolate randomly were tested in this study. The isolates were tested for susceptibility to clindamycin (2 µg) and erythromycin (15 µg) By Kirby Bauer disc diffusion method. Erythromycin (ERY) and Clindamycin (CLI) disks were placed 15-20 mm apart from each other on the plates.

Results: Of 302 collected S. aureus isolates, 134 (44.4%) were MRSA and 168 (55.6%) were MSSA. Inducible MLSB resistance, observed in 10% of all recovered MRSA and 3% of all MSSA isolates. The majority of MRSA isolates were constituted MLSB resistance (75%), this phenotype not seen among our tested MSSA isolates. Finally, 14% of MRSA isolates and 95% of MSSA showed sensitivity to Both ERY and CLI disks with large zones of inhibition around them.

Conclusion: Accurate susceptibility data are important for appropriate therapy decisions. Higher prevalence of MLSBi and MLSBc resistance in our MRSA isolates, also low prevalence of ERY-resistant and CLI-susceptible phenotype (MSB) among MSSA isolate suggested that performing the D-test routinely for S. aureus infections is not cost–beneficial and not recommended in our region.

Keywords: Staphylococcus aureus , clindamycin , Methicillin resistant

P111: **Detection of Mycoplasma Contamination in stem cells**

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Background and Aim: Stem cells are used in reasearches and cell therapy,The aim of this study was performed to detect frequent mycoplasma species in hair follicle stem cells culture (M. hyorhinis and M. arginini)with culture and PCR methods.

Methods: In this study young mouse hair follicle , human fetal shoan stem cells, Hela and vero cell lines were used , For detection of mycoplasma, First, the stem cells and cell lines were cultured 14 day after thawning, and supernatant of this cultures were taken after a culture period of at least 5days then supernatant of these cells culture are used for samples divided in 2 sections: first section was passed through 0.45µm pore-size filters, then inoculated into specific PPLO broth and agar media and incubated at 37°C under 5% CO2 in 7 days .culture method only used for detection of M.Arginini because M. Hyorhinis is non-cultivable , the second section was used for PCR in which primers were used for amplification of 16S rRNA gene of M. hyorhinis and M. Arginini.

Results: From a total of 8 samples tested, 0% were positive with culture and with PCR method for M. Arginini and 0% were positive with PCR method for M. Hyorhinis

Conclusion: Results of this study showed that no contamination in stem cells and cell lines with culture and PCR methods. Keywords: Contamination, Mycoplasma, Stem cells

Keywords: Contamination, Mycoplasma, Stem cells

P112: **Predictor of sternal wound Bacterial infection in patients undergoing open cardiac surgery in Isfahan Heart Center.**

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Background and Aim: Sternal Wound Infection (SWI) is an uncommon but potentially life threatening complication of cardiac surgery. Predisposing factor for SWI are multiple with varied frequencies in different studies. The purpose of this study was to assess the incidence , and risk factors after cardiac surgery at Isfahan Heart Center. According to the Centers for Disease Control and classifications, the infection of surgical wounds of sternotomies should be considered as (A) superficial if only the skin and subcutaneous tissue are involved, (B) deep when the infection reaches the sternum but does not involve it, and (C) organ /space when sterna osteomyelitis or mediastinitis occurs. This classification enables a better comparison of related research[3,4]. The incidence of mediastinal wound infection in patients undergoing median sternotomy and open heart surgery can be up to 5% [2]. A subgroup of 20-30% of those patients develops deep sternal infection with an associated morbidity, mortality and “cost” that remain unacceptably high[3].

Methods: This study prospectively evaluated multiple risk factor for SWI in 1346 patients who under went cardiac surgery at Isfahan Heart Center between Mar 2012 and Mar 2013. Cases of SWI were confirmed based on the criteria of the Centers for Disease Control and prevention (CDC). The parameters studied were: age, sex, cigarette smoking, hypertension, diabetes mellitus, length of preoperative hospital stay. Wounds were classified as per modified CDC's NNIS criteria.. Suspected sites of infection were cultured and antibiotic susceptibility of cultured organisms was tested[1

Results: In the study period, 1346 cardiac surgery were performed with a total SWI rate of 1.18 percent(16 cases). Patients were 52.2±21.59 years old. In multivariate analysis, 62.5% males (10 cases), 50% diabetics (8 cases),62.5% hypertension (10 cases), 68.75% length of preoperative hospital stay (11 cases), 37.5% cigarette smoking, 6.25% addiction (1 cases) were identified as significant predictors of SWI. Organisms isolated at sternum site were staphylococcus aureus 43.75% (7 cases), staphylococcus epidermis 6.25% (1 case), klebsiela 18.75% (3 cases), E. coli 12.5% (2 cases), Entrobacter 6.25 (1 case), Entrococci 6.25% (1case), pseudomonas 6.25% (1 case

Conclusion: Measures to reduce the high rates of SSI need to be instituted through a multidisciplinary effort including infection control education and specific SSI prevention activities at at Isfahan Heart Center.

Keywords: Infection ,mediastinitis, cardiac surgery

P113: **Reduction of vanA and vanB vancomycin resistance using Lactoferrin in vancomycin resistance enterococci (VRE) isolates**

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Background and Aim: Lactoferrin (LF) is a mucosal resistance glycoprotein that its antimicrobial effect was approved previously. In this study, prevalence of vanA and vanB resistant in vancomycin resistance enterococci (VRE) strains by phenotypic and Real time PCR methods was studied and the effect of LF on the vancomycin MIC of vanA and vanB vancomycin resistance isolates investigated using a modified simple and time-casting method based on E-test and compared with micro titer plate method.

Methods: Forty isolates of vancomycin resistant enterococcus were collected and identified using the standard biochemical tests and Real time PCR.

Results: Our results showed that 39 VRE isolates were E. faecium and one was E. faecalis. Phonotypic study by E-test method showed that 34 isolates had vanA phenotype (85%) and 2 isolates had vanB phenotype (5%). Whereas, the Real time PCR results showed that vanA gene was detected in 37 of 40 isolates (92.5%) and vanB genes was detected in 3 of 40 isolates (7.5%). Regarding vanA resistant isolate, reduction of vancomycin MIC in concentration of 256,512,1024, 2048µg/ml of LF in E-test modified method were 12.8,15.3,27.5 and 85 fold respectively whereas in microtiter plate method were 0, 3, 9.6 and 80 fold respectively. These results for vanB resistant isolate for same concentrations of LF in E-test modified method were 4.5,10, 8.2 and 8.2 fold respectively whereas in microtiter plate method were 8.8,10.3,4.3 and 4.5 fold respectively.

Conclusion: In all concentrations of LF, in E-test modified method better reduction in the MIC of vancomycin was observed comparing with microtiter plate method.

Keywords: E-test method, Lactoferrin, VanA, VanB, Vancomycin resistance enterococci (VRE)

P114: **Immunological evaluation of staphylococcus aureus conjugated vaccine**

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Background and Aim: Staphylococcus aureus is a subset of Staphylococcaceae can cause different disease in humans . The aim of present study is the preparation and immunological evaluation of S.aureus Capular type 8 - diphtheria toxoid (DT) conjugate in mice as a candidate vaccine.

Methods: Capsular type8 of this bacteria extracted by methanol sedimentation method. To improve immunogenicity, the purified antigen was coupled to DT with ADH as a spacer and EDAC as a linker. The reaction mixture was passed through a sepharose CL-2B column. Sterility and toxicity tests were shown that prepared conjugate was non-toxic and non-pyrogenic. Then four group of female BALB/c mice (each group was included 15 mice) was selected. Vaccination was performed by three doses with two week interval. Then serum samples was collected and antibody responses against Cap8 was measured by ELISA method for total IgG, IgG1, IgG2a, IgG2b, IgG3, IgM and IgA.

Results: Two week after first dose of vaccination there was no significance difference in antibody titers between groups that were immunizided by Cap8 and Cap8-DT. But after second and third doses, Cap8-DT showed significance increasing in all types of antibodies titers in versus Cap8. Overall results of anti Cap8 inductions for total IgG, IgM, IgA, IgG1, IgG2a, IgG2b, and IgG3 were shown: Cap8-DT>Cap8>DT. The anti Cap8 IgG was predominantly of IgG1 subtype and then IgG3, IgG2a, IgG2b, respectively.

Conclusion: These results indicated that Cap8 from S. aureus increase anti Cap8 in conjugate form with diphtheria toxoid and can be an appropriate effective candidate vaccine for this bacteria.

Keywords: Staphylococcus aureus, Conjugate, ELISA,Capsular type 8,Diphteria toxoid

P115: **Evaluating The ESBL Isolates In Clinical Escherichia coli In Karaj City, Iran**

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Background and Aim: Today, problem of drug resistance has become one of the era's greatest dilemmas. Restrictions on the number of effective antibiotics add to the importance of dedication to this issue. The city of karaj due to neighboring the capital city is over crowded with immigrants and This makes the problem of community acquired infections more serious.Since Urinary Tract Infection is one of the most common diseases, we decided toand evaluated the percentage of isolates producing broad-spectrum beta-lactamase enzyme in clinical samples obtained from UTI patients

Methods: 100 clinical E.coli sample were collected from medical centers in the city of Karaj. The isolates were cultured on differential and special media to ensure purity..beta lactamase producing isolates were detected by double disk synergy test method.

Results: from 100 isolates 33% (33) isolates were ESBL positive and producing broad-spectrum beta-lactamase enzyme .

Conclusion: a large number of isolates were ESBL positive. Appropriate Prescribing of antibiotic by clinicians would help the Infection control and prevent the spread of resistance.

Keywords: ESBL, E.coli,antibiotic resistance, Urinary tract infection

P116: **Determination of Plasmid Profile and Antibiotic Resistance Pattern in clinical Escherichia coli Strains in Karaj city**

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Background and Aim: In recent years the threat of acquisition of antibiotics resistance caused by over use of antibiotics is growing. The purpose of this study was to survey the pattern of antibiotic resistance and evaluate of Multi-drug resistance Escherichia coli and analyze the plasmid profile of these clinical strains.

Methods: This study was performed on 83 clinical Escherichia coli, obtained from four hospital and two private laboratories of Karaj city. Isolates were identified as Escherichia coli based on standard biochemical tests. The isolates were screened for Antimicrobial susceptibility test by 20 antibiotics using Kirby-Bauer disk diffusion method. Plasmid profiles of these isolates have been analyzed by alkaline lysis method.

Results: Antibiotic resistance pattern were observed as follows: Nitrofurantoin 8.4%, Tetracycline 63.8%, Amoxicillin 83%, Ofloxacin 30%, Levofloxacin 30%, Co-trimoxazole 59%, Amikacin 18%, Imipenem 25.3%, Choloramphenicol 16.9%, Cephalexin 61.4%, Gentamycin 28.2%, Norfloxacin 33.7%, Nalidixic acid 56.6%, Cephalothin 48.2%, Ciprofloxacin 32.5%, Ceftazidime 27.7%, Ceftriaxone 37.3%, Ceftizoxime 16.9%, Cefotaxime 38.5%, Amoxycillin/Clavulanic acid 68.7%. Of 83 isolates 92.77% (77) isolates were resistance to more than two unrelated drugs. Of 83 isolates, 65 isolates, (78.3%) showed the presence of plasmid

Conclusion: The results of this study indicate a growing resistance pattern to the first line antibiotics, prescribed for urinary tract infection. High presence of plasmids in these strains, play an important role in multidrug resistance in these bacteria

Keywords: Antibiotic resistance, Escherichia coli, Multi-drug resistance, plasmid

P117: **The survey of pneumococcal nasopharyngeal carriage and the risk of colonization in students in kashan**

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Background and Aim: S.pneumoniae is known as an important pathogen that cause serious infections such as: sepsis, pneumonia and meningitis. Nasopharyngeal colonization is recognized as a first step in infections caused by S.pneumoniae. The aim of this study was to determine the prevalence of nasopharyngeal colonization by S.pneumoniae and the related risk factors in students in Kashan.

Methods: A descriptive study was conducted in 1289 students in Kashan during Aban 1390 to Azar 1391. A questionnaire including questions about clinical background and demographic characterization was filled by student's parents. Samples were cultured on selective media. S.pneumoniae strains were recognized by gram staining, detecting alpha hemolysis, catalase, optochin susceptibility and bile solubility tests.

Results: Participants ranged from 7 to 19 years old (mean age13.08± 3.246) and 55.8% (719) of them were male and 44.2% (570) of them were female. Prevalence of S.pneumoniae nosopharyngeal carrier was 14% (181). Significant relationship was seen between sex, age, previous respiratory infections, asthma, runny nose, previous hospitalization and increasing rate of pneumococcal carriers.

Conclusion: According to high prevalence and significant relationship between increasing rate of pneumococcal colonization and sex, age, previous respiratory infections, asthma, runny nose and previous hospitalization, employing precaution strategy such as student vaccination, is necessary.

Keywords: Streptococcus pneumonia, nasopharynx carrier, student

P118: **Antibiotic resistance pattern and distribution of blaNDM and blakpc genes among Acinetobacter baumannii isolated from patients in hospital of Tehran**

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Background and Aim: In recent years, Acinetobacter baumannii is one of threatening microorganisms for curing through antimicrobial drugs, because of its clinical effect and its ability of drug resistance. On the other hand, infections with bacteria which produce extended-spectrum beta-lactamases (ESBL) increase the above problem in worldwide. So the purpose of this study was to define antibiotic resistance pattern and distribution of blaNDM and blakpc genes in A. baumannii isolates which isolated from patient in hospital of Tehran.

Methods: This study is done on 500 clinical samples in 3 hospitals in Tehran during one year. After identification of isolates in species level using cultural and biochemical methods, the susceptibility tests were carried out on 100 isolates of A. baumannii using disk diffusion method for 11 different antibiotics according to the Clinical and laboratory Standards Institute (CLSI) guidelines. Then isolates were considered for presence of blaNDM and blakpc genes by PCR

Results: In this study 100 isolates of A. baumannii and 20 isolates of others Acinetobacter were isolated from patients. More than 55% of isolates showed multiple-drug resistance and also above 90% resistance to cefepime, ceftriaxone, and amikacin was recorded. The PCR results showed that 19 cases (19%) and 13 cases (13%) of isolates had blaNDM and blakpc genes respectively which most of them had been isolated from patients who were hospitalized in the ICU

Conclusion: Multiple-drug resistant A. baumannii is expanding in Iran and it is considered as an important hazard for hospitalized patients. Moreover regarding to existence of blaNDM and blakpc genes in this bacterium and possibility of transformation of these genes to the other microorganisms, reconsideration in antibiotics consumption patterns and more attention to nosocomial infections control criteria are inevitable.

Keywords: Acinetobacter baumannii, multiple-drug resistance, blaNDM and blakpc, PCR

P119: **Frequency of ESBL-producing strains of Klebsiella pneumoniae isolated by two phenotypic and genotypic methods in Qazvin, Iran**

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Background and Aim: Klebsiella pneumoniae is an opportunistic pathogen causing nosocomial infections, such as urinary tract infections, pneumonia, septicemia and soft tissue infections. Several studies have shown that ESBL- producing Klebsiella pneumoniae is rapidly increasing worldwide. The aims of this study were (i) to determine the antimicrobial susceptibly of Klebsiella pneumoniae isolates (ii) to assess of the ESBL-producing isolates using the phenotypic and molecular methods.

Methods: In this study, from May 2011 to June 2012, 149 Klebsiella pneumoniae isolates were collected from patients in Qazvin and Tehran hospitals. All isolates were initially screened for ESBL-production by disk diffusion method according to CLSI guideline and then were confirmed by a combination disk method. ESBLs-production isolated were subjected to polymerase chain reaction (PCR) for detection of ESBLs genes including blaTEM and blaSHV.

Results: Of 149 isolates, 93 isolates (62.41%) showed the reduction of susceptibility to the screening antibiotics. Among them, 75 isolates (50.34%) were positive for ESBL production. blaTEM and blaSHV were detected in 41 isolated (54.66%) and 29 isolates (38.66%), respectively. Five phenotype ESBLs-producing Klebsiella pneumoniae isolates were negative for any of the ESBLs genes.

Conclusion: Considering the high frequency of extended spectrum β-lactamases producing isolates in the selected hospitals, initial identification and following of them are necessary to prevent more spread in the hospitals. The appropriate treatment methods and the rational use of antibiotics are also important.

Keywords: Klebsiella pneumoniae, extended spectrum β-lactamases, blaTEM ،blaSHV

P120: **In vitro antifungal activity of Clotrimazole, Miconazole and Ketoconazol by binary mixture pattern against hospital isolates of Candida**

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Background and Aim: The lack of variation in antifungal drugs, and inappropriate use usually causes resistant strains of the yeast in human's normal flora. Unfortunately, a large number of Candida infection cases in immunosuppressed patients with insufficient treatment eventually can cause patient's death. The aim of this study was to evaluate the in vitro conventional antifungal azole compounds with binary mixture with appropriate ratios by susceptibility testing using the laboratory as a mixture of two in vitro conditions.

Methods: In present study, 10 isolates of Candida were admitted from patients which were referred to mycology laboratory of Faghihi Hospital at Shiraz with signs of cutaneous and mucosal infections. we use the methods of the binary mixure of common antifungal drugs Clotrimazole, Ketoconazole and Miconazole on equally proportion can be solved in various concentrations on the SDA medium and then Cndida isolates can be cultured in the SDA plates. The minimum inhibitory concentration (MIC 90) and minimum fungicidal concentration (MFC) was determined for each individually.

Results: Survey results suggest that a binary mixture of these drugs in most strains inhibited the growth of pathogenic Cndida isolates. The combination with Clotrimazole and ketoconazole in equal proportion had more effective than other drug mixtures aganist all isolate with the exception of isolate 3, the lowest MIC was range, 0.39 to 6.25 µg/ml. contrary, the combination with Miconazole and Clotrimazole had the least effect on each other, and the MIC was calculated in the range of 3.12 to 50 µg/ml. Evaluation of MFC showed almost the same results. Lowest values of the MFC belonged with the combination clotrimazole and ketoconazole and were obtained 6.25 µg/ml.

Conclusion: the MFC values of Clotrimazole and individually Ketoconazole were obtained average ranges in 25-50 µg/ml. Analysis of the antifungal activity of Miconazole, individually or in binary mixture with the other azole drugs did not decrease drugs concentration significantly. As the MIC and MFC average of Miconazole in single mode was 0.39 and 50 µg/ml respectively. The MFC of combination with Miconazole and Ketoconazole in equal proportions were obtained 50 and 100 µg/ml, respectively, 50% and 40% frequency, which is even more than a single state. according to these results, we can conclude that the use of drugs the combination Clotrimazole and Ketoconazole in equal ratios, when compared with a single mode in the same amount is shown of better antifungal effect agaist cutaneous and mucosal isolates of the Candida infection.

Keywords: Candida , Clotrimazole, ketoconazole, MIC, MFC

P121: **Assessment of a Loop Mediated Isothermal Amplification(LAMP) Techniques for the Detection of Adenovirus Keratitis**

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Background and Aim: Adenovirus Keratitis is an important factor with human. So quick and timely detection of adenovirus keratitis to prevent the spread of disease and the appropriate treatment of the major issues in the diagnosis of this virus. Virus detection methods (serological and molecular) each have limitations, So you can use them easily and diagnostic centers exist at all. This study is based on molecular techniques to LAMP, the virus is suspected in a patient, careful, fast and easy to recognize.

Methods: In this study, 86 suspected patients to Keratitis referred to Farabi Hospital have been examined . DNA samples of patients have been extracted using boiling and DNG-Plus. Then, specific primers have been designed for LAMP technique. First, sensitivity tests have been done for LAMP test and then optimized for the samples. At the end, LAMP production have been examined adding cyber-green

Results: The LAMP test is optimized thank to extracted DNA from Adenovirus. It was specified in sensitivity test that suggested sensitivity is about one particle per virus and no result was obtained during specification test by any of tested DNA. the results of 28 samples out of 86 (%32) with specific particle and different titers were positive.

Conclusion: Using LAMP technique to diagnose adenovirus showed that isothermal proliferation technique by loop (LAMP) has more sensitivity and precision. In addition, this technique provide a faster and more trusting method for diagnosing infections such as Keratitis that there is an emergency need to be diagnosed to be cured.

Keywords: Adenovirus, LAMP,Keratitis, DNA

P122: **Prevalence of serologic markers hepatitis B viruses in special patients in mazandaran**

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Background and Aim: Hepatitis is a common disease placed in liver of human and proliferates. Produced substances by this viruse pour in to blood. Doing sensitive blood tests define viruse proliferation in a body. This viruse is a serious danger to transfer in special patients (Thalasemia, Dialysis, hemophilia) And totally in patients who receive blood repeatedly. The aim of this study is definition of different HBV serologic markers in special patients in the way of serology in Mazandaran.

Methods: From the total of 94 serum samples in special patients clienteles to clinics of Mazandaran in the first 6 month of 1391, samples were collected and identified by the way of ELISA and were evaluated by using SPSS Statistical software.

Results: Evaluating HBV serologic markers in these special patients about the number of HBs Ab title was (57.4%), but HBe Ab (12.6%), HBc IgM (13.8%), HBc total were (10.5%) respectively. According to accomplished test the errant of HBs Ab in comparison of another test had a meaningful difference ($p<0.05$). The result of anti-HIV is negative, but anti HCV ispositive.

Conclusion: The finding of this study showed that the most antibody title related to HBs Ab and considering all these patients receive vaccine, in negative people it was caused by no repeatation the vaccine reminder and because these people are in dangerous group, should be concerned.

Keywords: HBV, Thalasemia, Hemophilia, Dialysis, ELISA, Vaccine.

P123: **Study of onychomycosis in patients with azad medical mycology lab university of sari(1389-91)**

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Background and Aim: onychomycosis common fungal infection of nails, hands and feet, which is a kind of fungi, and yeast like fungi on seeds may be saprophytes.would have.

Methods: Nail chips with 20-10% KOH were transparent and were examined microscopically. Well as some of the chips in environments Sabouraud dextrose agar (SDA) and dextrose agar with chloramphenicol and cycloheximide (Scc) were planted.

Results: Among 75 patients with lesions of the nails in the hands and feet of 27 patients (4/37%) were diagnosed as mycosis typically infaction. A total of 18 patients (8/64%) were female and 9 (2/35%) and male sex. Among the fungi isolated in 19 cases (2/68%)yeast and yeast like, 5 (3/19%), dermatophytes and 3 (5/12%) were the saprophytic fungi. Candida albicans, Trichophyton Mentagrophytes and Scopulariopsis than other fungi were isolated as onychomycosis factors.

Conclusion: The results of this study indicate that onychomycosis of fungal infections in patients with lesions of the nail.yeast And yeast like fungi causing the infection factor more. The children also suck for nails, scratching the anal area are regarded as causes of disease. Seems to Candida albicans, Trichophyton Mentagrophytes and Scopulariopsis most common factor onychomycosis in this area.

Keywords: onychomycosis, dermatophytes, saprophytes, like yeast, Candida albicans

P124: **Molecular epidemiology and characterization of virulence and antimicrobial resistant genes of community acquired and hospital acquired methicillin-resistant Staphylococcus aureus isolates in West Iran:**

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Background and Aim: Methicillin-resistant Staphylococcus aureus (MRSA) is a pathogen of public health importance.To date, little is known about the prevalence of MRSA and their antimicrobial resistant and virulence genes and common clones in the community and hospitals in Western of Iran. We aimed to investigate antimicrobial resistant profiles and their encoding genes and different virulence determinant and to find common SCCmec types and spa types among HA and CA-MRSA isolated from West Iran.

Methods: Totally 500 isolates suspected to Staphylococcus spp., were collected from different hospital of West Iran during 2010-2012. We used phenotypic and genotypic methods for identification of bacteria as specie level. Antimicrobial susceptibility of 12 antibiotics was done by DAD according to CLSI guidelines. We used specific primers for detection of 21 different resistant genes and virulence determinates including exotoxin (et), Hemolysins (hl) and enterotoxines (ea) of all isolates. Then, we performed PCR-sequencing of spa types and SCCmec types according to previously report methods.

Results: 100% of isolates harbored mecA and spa and classifieds as MRSA. 100% of MRSA was resistant to oxacillin-screening disc method. 80% of MRSA isolates had femA. Samples was recovered from wound: 49% , blood: 32%, urine: 14%, sputum: 5%, tracheae: 1% respectively. 38% of isolates recovered from wounds of outpatients and classified as CA-MRSA and others 62% recovered from nosocomial infections and named HA-MRSA. All isolates were susceptible to synercide, linezolid and vancomycin, but were resistant to penicillin (100%), erythromycin (50%), clindamycin (27%), ciprofloxacin (23%), tetracycline (23%), rifampicin (14%), tigecycline (1%), and imipenem (1%) respectively. All beta-lactam resistant isolates harbored blaZ. Nearly all tetracycline and erythromycin resistant MRSA had ermA and/or erm C but not erm B. three erythromycin resistant isolates had msrA. LinA was detected in 16 isolates but only four clindamycin resistant harbored this gene. Pvl was detected only in 4% of isolates belonging to HA-MRSA. The prevalence of different exotoxin among these isolates were tst (46%), etA (3%), etB (1%), hlg (73%), hld (73%), hla (64%), hlb (7%), sea (41%), sec (14%) and seb (2%) respectively. 13 different spa types and 4 SCCmec type and subtypes was identified. In SCCmec typing most of isolates in HA and CA- MRSA belonged to IIIA (41%), IVc (20%), IVd (13%) and V (3%) respectively. The most spa types were t701 (30%), t12311 (15%) and t021(11%). T12311 was identified among HA and CA-MRSA for the first time in Iran and throughout the world.

Conclusion: This is the first large scale study on molecular epidemiology of HA and CA- MRSA in West of Iran. During this study we identified new spa type t12311 for the first time in the word. This spa types have two SCCmec type IIIA and IVc and different antimicrobial phenotypes. Finally we repot emergence of SCCmec type IIIA and spa types t701, t2651 and t12311 with different phenotypic and genotypic determinants in west of Iran.

Keywords: Antimicrobial resistant- virulence determinants- SCCmec type- SPA type

P125: **Prevalence of Streptococcus pneumonia in patients diagnosed with pneumonia by culture and PCR**

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Background and Aim: Pneumonia is an acute infection of the lower respiratory tissues accompanied by signs and symptoms and evidence of chest infiltration on x-ray or altered breath sounds on auscultation. Depending on their co-morbidities, residents may develop community-acquired, end-of-life or aspiration pneumonia. Streptococcus pneumonia (pneumococcus) is responsible for approximately two-thirds of bacterial community-acquired pneumonia cases as well as the most deaths from pneumonia, particularly in the elderly. The risk is highest in individuals with diminished immune competence, smokers and those with chronic conditions including cardiovascular or pulmonary disease, and diabetes.The aim of this study was to determine the bacterial etiology in adult patients with .pneumonia infection by implementing polymerase chain reaction. A total of 126 cases (45 were male and 81 were female; age range: 1-80 years, mean age: 44) who were admitted to our hospital and clinically diagnosed as pneumonia between November 2011 - June 2012, were included in the study. Respiratory samples (sputum and blood) obtained from patients were searched by PCR method (Sinagen companies) in terms of the presence of Streptococcus pneumonia. The samples were simultaneously inoculated onto 5% sheep blood agar. The bacterial etiology was identified in 63 (50%) of 126 patients with .pneumonia and a total of 73 pathogens were detected. The leading organism was S. pneumonia. It was concluded that PCR/RLBH method supplemented the determination of bacterial etiology in our study cases by S. pneumonia.

Methods: After completing the questionnaire, patient tracking numbers of 126 sputum samples collected and then Gram stain, culture, and PCR were performed.

Results: Of the126 samples, 35.7%were male and 64.3% female mean age was 44 years. A number of 45 patients were positive whereas the results for PCR were showed positive for 63 patients.

Conclusion: Theresult of this study has shown the importance of streptococcus pneumonia in the society and because PCR is a fast method for detection of bacteria and can have good results in treating pneumonia before wasting time.

Keywords: streptococcus pneumonia, culture, PCR , pneumonia.

P126: **Prevalence of Legionella pneumophilla in patients diagnosed with pneumonia by PCR**

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Background and Aim:: One of the most common factors in the incidence of nosocomial infections and most Legionella pneumophilla is a gram-negative bacterium from Legionellosis is caused by the disease.Bacteria through the air particles to move through the water resources are being published and will involve the respiratory system.Hospital environments in terms of growth and transport of contaminated particles to place people at risk with high potential for growth and spread of these bacteria,The ventilation and cooling systems in recent years has caused the outbreak, Given these problems, and due to increasing cooling systems in most places we need a fast and reliable method for identifying need

Methods:: After positioning the patient and complete the questionnaire and 126 blood samples collected sputum samples for Gram stain and PCR was performed.

Results: Of the 126 samples, 35.7% men and 64.3% female and their average age was 44 years. Including chest pain 84.9%, 96% phlegm, dyspnea 87.6% and 46.6% had a history of respiratory infections, results PCR 13 patients (10.3%) were positive.

Conclusion: The results of this study coincide with clinical symptoms confirming the positive PCR Resources Legionella pneumoniae were caused by this bacteria can be important in terms of research and realized that PCR is a fast method to detect pneumonia can have good results in treating pneumoniae prior to wasting time.

Keywords: Legionella pneumophilla , RCR , pneumonia

P127: **Antimicrobial activity and chemical composition of the essential oil of Chamaecyparis lawsonian by macrodilution method and Gc mass analysis**

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Background and Aim: Increasing in the number of multidrug resistant bacteria is a big challenge in different infectious disease treatment,leading to search for new drugs specially with natural sources.Chamaecyparis lawsoniana is a native tree of north America which has been used as traditional medicine by Indian American tribes

Methods: In this study ,the aerial parts of C.lawsoniana were used for producing the essential oil.The Eo was investigated for its antibacterial and antifungal activity against 4 bacteria and 1 fungi.The effective chemical part of the essential oil was recognized by bioautography and then analyzed by Gass chromatography mass spectrum. In this study ,the aerial parts of C.lawsoniana were used for producing the essential oil.The Eo was investigated for its antibacterial and antifungal activity against 4 bacteria and 1 fungi.The effective chemical part of the essential oil was recognized by bioautography and then analyzed by Gass chromatography mass spectrum. In this study ,the aerial parts of C.lawsoniana were used for producing the essential oil.The Eo was investigated for its antibacterial and antifungal activity against 4 bacteria and 1 fungi.The effective chemical part of the essential oil was recognized by bioautography and then analyzed by Gass chromatography mass spectrum. In this study ,the aerial parts of C.lawsoniana were used for producing the essential oil.The Eo was investigated for its antibacterial and antifungal activity against 4 bacteria and 1 fungi.The effective chemical part of the essential oil was recognized by bioautography and then analyzed by Gass chromatography mass spectrum. In this study ,the aerial parts of C.lawsoniana were used for producing the essential oil.The Eo was investigated for its antibacterial and antifungal activity against 4 bacteria and 1 fungi.The effective chemical part of the essential oil was recognized by bioautography and then analyzed by Gass chromatography mass spectrum. In this study ,the aerial parts of C.lawsoniana were used for producing the essential oil.The Eo was investigated for its antibacterial and antifungal activity against 4 bacteria and 1 fungi.The effective chemical part of the essential oil was recognized by bioautography and then analyzed by Gass chromatography mass spectrum.

Results: The essential oil showed the antibacterial effect against Staphylococcus aureus, Bacillus cereus, Escherchia coli and Candida albicans by 1.56 mg/ml, 1.56mg/ml, 3.125mg/ml, 3.125mg/ml MIC value. The essential oil had no effect on Pseudomonas aeroginosa. Gc mass analysis of Eo revealed that the major components and the percentage of the effective part isolated by bioautography were monoterpenes hydrocarbons 47.2%, oxygenated monoterpenes 45.1%,oxygenated sequiterpenes 4.5%.

Conclusion: Our data clearly showed that the Eo of C.lawsoniana has the effective antibacterial and antifungal activity and can be used for further investigation as a potential antibiotic.

Keywords: Antibacterial activity,Chamaecyparis lawsoniana,Gass chromatography, Essential oil

P128: **Quantitative Real Time PCR assay for determining cap copy numbers in H influenzae b isolates and its relation to capsular polysaccharide production**

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Background and Aim: The capsular polysaccharide of Haemophilus influenzae serotype b [(3)-beta-D-ribose-(1-1)-ribitol-5-phosphate] is a major virulence factor and a target for serum antibodies which protect individuals against invasive infections. The capsule locus is 18kb in size. Within most invasive Hib isolates there is cap b duplication of a 17 kb region organized as direct repeats separated by a smaller (1-2 kb) region of non-repeated DNA. Homologous recombination between the direct repeats is rec dependent and results in high-frequency loss of capsule expression and virulence. Owing to the importance of cap copy number to PRP concentration in Hib isolates an important goal of the study was set to determine cap copy number in these isolates using Real time PCR. The results were then compared to PRP concentrations and virulence in these isolates.

Methods: Four local Hib isolates were selected for studies. A quantitative real time PCR assay was used to determine the cap copy number in these isolates. 3 sets of primers including rpoB gene single copy gene, IS1061 and Hib specific primers were used. The number of copies of cap or IS1016 was calculated from the ratio of the amount of Hib specific or IS1016 product to rpoB product, respectively. PRP concentration in these isolates were determined and compared with Real time PCR assay results.

Results: In two clinical type b strains, the capsulation locus had a single copy of this 17-kb segment. In addition, three copies were detected in one of the isolate. On comparing capsule polysaccharide production by these three type b strains with that by a prototypic type b strain with a duplicated locus, a gene dosage effect was demonstrated, with a halving of detectable polysaccharide in the single-copy strains. According to Bial assay results, a three-copy strain made about two times more capsular polysaccharide than did an isogenic one-copy derivative. The evolutionary significance of the duplicated arrangement may be its ability to rapidly amplify under conditions where it is advantageous to produce more capsules. Despite this reduction in polysaccharide, these strains retained virulence potential as evidenced by bacteremia and meningitis in infant rats. As well as sub serving augmented capsule polysaccharide production, a duplicated configuration of the type b cap locus endows strains with genetic instability not found in capsulate single-copy variants.

Conclusion: We speculate that a survival advantage might be conferred on Hib strains carrying duplication at this locus as a result of gene dosage, the genetic instability of the locus, or both. Use of real time PCR in the detection and quantification of cap genes has important clinical implications. The assay could also be utilized for monitoring changes in virulent serotypes of H influenzae other then type b.

Keywords: cap gene, H.infleunzae b, PRP, Real time PCr

P129: **Prevalence and Risk Factors for colonization of Vancomycin-Resistant Entrococci (VRE) in Patients in The Intensive Care Unit Running title: Colonization of VRE in ICU**

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Background and Aim: The aim of this study was to determine the prevalence of VRE strains isolated from rectal swabs of hospitalized patients in ICU.

Methods: This cross sectional study, done in the Beheshti hospital on 156 rectal swab samples were collected in 3 departments of intensive care unit from November 2011 to April 2012. Antibiotic sensitivity test was done by Kirby-Bauer method. Comparison between groups was performed by using chi-square and Fisher test.

Results: Enterococcus was detected in 135 out of 156 (86.5%). The prevalence of VRE was 42.9%. Of the 58 VRE isolates, 46 (79.3%) , 10 (17.2%) and 2(3.5%) samples had Van A, Van B and Van C phenotypes , respectively. 55 out of 58 (94.8 %) patients had previous usage of antibiotics (P=0.037) and 54 out of 58 (93.1%) patients had usage of three to four types of antibiotics (P=0.009). There was a significant correlation with the number of hospitalize days. A significant association was not observed between VRE and use of corticosteroids, the diabetes, history of hospitalization.

Conclusion: Prevalence of VRE was observed 3.6 times more in patients who had taken antibiotics. Also, with increasing number of antibiotic consumption of multiple types, risk of antibiotic- resistant enterococci increases 2.65 times

Keywords: Vancomycin-Resistant entrococci - Risk factors - Intensive Care Unit

P130: **Nasopharyngeal Carriage of Streptococcus pneumoniae in Healthy school-age Children: Serotyping, antibiotic susceptibility and related risk factors**

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Background and Aim: Streptococcus pneumonia is an important problem worldwide and nasopharyngeal colonization plays significant role in pneumococcal infections. The aims of this study were to determine the nasopharyngeal colonization rate, serotyping, antibiotics susceptibility and study the risk factors for nasopharyngeal colonization with S. pneumonia from students in Kashan, Iran.

Methods: All children enrolled in this study were sampled in December 2011 to November 2012 in 22 schools in Kashan, Iran. We excluded children showing symptoms or signs of RI, as well as those who received at least one dose of any antibiotic treatment during the previous 15 days. One of the children's parents signed informed consent and provided clinical and demographic information, such as age, sex, crowding (i.e., two or more people sleeping in the same room), siblings, smokers at home, and socioeconomic status. Detailed medical information was recorded, such as previous diagnosis of asthma, allergic rhinitis and history of hospitalization in the previous 6 months, and antibiotic treatment in the previous 3 months. The study was approved by the ethical committees of Kashan University of Medical sciences. Specimens from nasopharynxs of the child were obtained using a sterile cotton-tipped swab. Swabs were plated immediately onto brain heart infusion agar plates with 5% blood and 4 μg/ml of gentamycin. S. pneumoniae was identified by Gram stain, colony characteristics, susceptibility to optochin, and bile solubility. S. pneumoniae strains were investigated for the presence of the most common pneumococcal serotypes using a multiplex polymerase chain reaction (PCR) assay

Results: A single swab was obtained over the nasopharyngeal walls of the 2100 healthy students, of which 291 (13.9%) were found to be carriers. The carrier rate was significantly higher in the 12 to15 old age group. Upper respiratory tract infection within the last month (OR=1.5, p<0.011), previous hospitalization (OR=1.6, p<0.001), previous antibiotic usage last two weeks (OR=1.89, P<0.001), rhinorea (OR=1.9 p<0.001), male sex(OR=3.5 p,0.001) and passive smoking (OR=1.56, p< 0.001) have been determined to be risk factors for S. pneumoniae carriage. The most prevalent serogroups were 19F, 6A/B, 15A, 11, 23F,1 and 35B.

Conclusion: The highest rates of S. pneumoniae colonization were observed among children with age 12-15 years old (15.8%), male sex (18.8%), rhinorrhea(21.4%) and with previous respiratory infection (18.9%).

Keywords: Streptococcus pneumoniae, colonization, serogroup

P131: **Frequency of Neisseria gonorrhoeae endocervical infection among female patients and Changing trends of Antimicrobial Susceptibility patterns in Kashan, Iran**

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Background and Aim: Neisseria gonorrhoeae is one of the most general sexually transmitted diseases in developing countries, and the emergence of resistance to antimicrobial agents in gonococci is a chief problem in the control of gonorrhea. The aim of this study was determination of endocervical gonococcal infection and antibiotic susceptibility in Kashan, Iran.

Methods: In this descriptive study, 294 endocervical swabs were collected from the obstetrics and gynecology clinics between December 2012 to May 2013 of the Kashan, Iran. The samples were cultured in modified Thayer Martin in 37° C with 5% CO2 for 48 h. Gram staining, oxidase, catalase and carbohydrate utilization test were used to confirm the isolated species. All the isolates were tested for antimicrobial susceptibility using the Kirby Bauer-disc diffusion techniques.

Results: The overall risk of gonorrhea was 2.38% (95% confidence interval [CI] 1.5-3.26%). All isolates were resistance to ceftriaxone, penicillin G, ciprofloxacin, tetracycline, cefepime, except for 2 isolate that was intermediate to tetracycline.

Conclusion: The present study emphasizes the importance of surveillance of antimicrobial resistance of N. gonorrhoeae in order to supervise the rate of multi resistant strains and to revise the cure recommendations.

Keywords: Neisseria gonorrhoeae, antimicrobial resistance, endocervix

P132: **The prevalence and patterns of antibiotic resistance among uropathogens referred to Kashan central laboratory**

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Background and Aim: Urinary tract infection (UTI) is the most common infection in both community and hospital patients. In majority of the cases, empirical antimicrobial treatment is practiced before the laboratory results of urine culture. Thus, antibiotic resistance may increase in urinary bacterial pathogens due to improper use of drugs. A prospective surveillance study was conducted to investigate the prevalence and patterns of antibiotic resistance among uropathogens from patients referred to Kashan central laboratory.

Methods: This study was conducted on 418 patients in Kashan, Iran, from April 2011 to October 2012. Bacterial species identification, antimicrobial susceptibility testing was performed for relevant bacterial isolates. Antimicrobial susceptibility test was performed for the isolates by Modified Kirby-Bauer disk diffusion method.

Results: Patients ranged from 1 to 91 years old (mean age 45.30 ± 2.381 years) and 58 out of 418 patients (13.9%) were males and 360 (86.1%) females. Analysis of urine samples from 6460 patients yielded 418(6.5%) bacterial isolates. The most frequent causative organisms isolated were Escherichia coli 336 (80.4%), klebsiella 49 (11.7%), Coagulase Negative Staphylococci 28 (6.7%), enterobacter 1 (0.2%), pseudomonas 2(0.5%) and proteus 2(0.5%). Nitrofurantoin and imipenem were found to be the most effective antibiotic against gram negative bacilli. The antibiotic resistance against Gram negative bacilli was found out is amoxicillin/clavulanate 84.8 %, ampicillin (76.6%), trimethoprim/sulfamethoxazole(65.8%), cephalotin 61.5%, ofloxacin 41.6%, ciprofloxacin 40.1%, imipenem (29.3%), and nitrofurantoin (13.5%).145 out of 336 E. coli (43.2%), 17 out of 49 strains klebsiella (34.7%) and 15 out of 27 strains Coagulase Negative Staphylococci(55.6%) were multi drug resistant. The most resistant of Coagulase Negative Staphylococci were to oxacillin (84%), erythromycin (72.2%), clindamycin (58.3%) and ampicillin (56%).

Conclusion: The results represent the increasing antibiotic resistance against microorganisms among the community-acquired UTI patients in Kashan, Iran. More than 80% of Coagulase Negative Staphylococci were resistant to meticillin. So, the physicians should consider resistance status of the infectious agent and choose effective antibiotics which are nitrofurantoin and imipenem for their empirical antibiotic treatments.

Keywords: urinary tract pathogen, antibiotic resistance, prevalence

P133: **Title: Frequency of erythromycin resistance phenotypes in streptococci isolates collected from laryngoscope in Shahid Rajaei hospital, Qazvin .**

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Background and Aim: Background&Aim: Macrolide (erythromycin) resistance in streptococci isolates may have constitutive or inducible resistance to lincosamide (clidamycin).In phenotypes in streptococci isolates. IN this study we determind the all erythromycin resistance phenotypes in streptococci isolates.

Methods: materials and methods: Twenty three streptococci isolates were subjected to routine susceptibility testing including erythromycin (E: 15µg) and clidamycin (CD: 2 µg) by kirby bauer disc diffusion method . Inducible clidamycin resistance was detected by D-test in erythromycin resistant isolates according to CLSI guideline.

Results: Results: Intotal , One isolate (4.35%) showed inducible clidamycin resistance , 6 isolates (26.1%) showed constitutive resistance, 5 isolates (21.73 %) showed MS phenotype ,while remaining 11 isolates (47.82%) showed wildtype (sensitive to both of them).

Conclusion: Conclusion: The results of this study showed that laryngoscope potentialy can be carried macrolid and clidamycin resistance isolates . So , using the appropriate infection control strategy before application of laryngoscope would be nescessary for managing of admitted patients.

Keywords: Keywords: Laryngoscope, streptococci , D-test

P134: **Investigated synergistic interaction among CM11 hybrid antibacterial peptide and clinical antibiotics by Time killing assay on Staphylococcus aureus and Pseudomonas aeroginosa**

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Background and Aim: Over the time, various mechanisms have been acquired by bacterial pathogens in order to deteriorate (neutralize, undermine) impacts of different antibiotics and effectiveness of older agents have continuously been reduced. Antimicrobial peptides are key components of native immunity. During recent years novel peptide antibiotics have been developed. CM11 peptide (WKLFKKILKVL-NH2), a short cecropin–melittin hybrid peptide, is an 11 amino acid amphipathic peptide with potent antimicrobial activity against Gram-positive and Gram-negative organisms, including antibiotic-resistant strains. In this study, we assessed in vitro activity of CM11 in combination with clinically used antimicrobial agents against several strains of P. aeroginosa and S. aureus, isolated from tow hospital of Tehran (Khatam al anbia and Shahid motahari) by the standard Time Killing assay method according to NCCLS protocols.
Methods: In interaction studies, some of the more resistance strain of each bacterium (on the base of their MIC and MBC) selected and were used to test the antibiotic combinations by a Time killing assay. In this method tubes containing freshly prepared Mueller-Hinton broth supplemented with the drug were inoculated with bacteria isolates to a density of ~5 × 105 CFU/ml in a final volume of 10 ml and incubated in a shaking bath at 37°C. Aliquots were removed at time 0, 1, 2, 3, 4, 5 and 6 h post inoculation, and serially diluted in saline for determination of viable counts. Diluted samples (100 µl) were cultured on Mueller Hinton agar plates and bacterial counts were determined after 18h incubation at 37°C. The antibiotic concentrations used in time-kill assays corresponded to the MIC values in combination as determined by the chequerboard method. Synergy was interpreted as ≥ 2 log10 decrease in CFU/ml by the drug combination when compared with its most active constituent, and = 2 log10 decrease in the CFU/ml below the initial inoculums, at any time point. The drug combination was considered to be antagonistic for = 2 log10 increase in CFU/ml and indifferent for < 2 log10 change in CFU/ml. All synergistic interactions were confirmed by triplicate assays.
Results: In the combination studies, no signi?cant differences were detected between Gram-positive and Gram-negative organisms. Synergy was observed when the peptide was combined with Penicillin, Oxacillin, Gentamicin, Rifampicin, Norfloxacin and Ciprofloxacin against S.aureus. Also synergism intraction was observed when the peptide was combined with Ceftazidime, Norfloxacin, Ciprofloxacin against P. aeroginosa but no intraction observed when Gentamaicin and Amikacin used in combination with CM11 Peptide against p. aeroginosa.
Conclusion: The mechanisms of synergy are complex and specific, and it is only now that they are beginning to be understood. The synergistic interaction with clinical antibiotics could be due to their increased passage through the bacterial membrane. In fact, the cationic peptides allow maximal entry of several substrates into the cell. Further studies are needed to elucidate the molecular mechanisms responsible for synergistic interactions. It will also be necessary to combine in vitro findings with additional pharmacokinetic and pharmacodynamic data in order to provide more meaningful prediction of the in vivo efficacy of synergistic combinations in clinical practice.

Keywords: Antimicrobial peptide, Staphylococcus aureus, Pseudomonas aeroginosa, Synergism, Time Kill assay

P135: **Investigation of synergism effect the CM11 peptide in combination with clinical antibiotic agents using against multidrug-resistant strains of E. coli, K. pneumoniae and A. baumannii**

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Background and Aim: In the last decade the rapid spread of antibiotic resistance among nosocomial bacterial strains is a major concern. So, in recent years, much attention and effort has been paid to search for new classes of antimicrobial compounds with new mechanisms of action. Among the large number of compounds that are currently under investigation for infection therapeutic, antibacterial peptides (AMPs), are promising candidates as a novel therapeutic agents with a unique mechanism of action. Antimicrobial peptides have been isolated from a variety of sources, including insects, mammals, amphibians, and plants and play important roles in the host defense system and innate immunity. The aim of this study was to investigate the synergistic effects of CM11 peptide (WKLFKKILKVL-NH2), a short cecropin–melittin hybrid peptide and common clinical antibiotics against three multidrug resistance bacteria, Escherichia coli, Klebsiella pneumoniae and Acinetobacter baumannii, to reduce the dose usage of peptide and antibiotics and subsequently, decrease the associated side effects.

Methods: Antimicrobial activity of CM11 was measured by MIC, MBC in previous our group studies and in new studies we used of chequerboard titration method to combination studies. In interaction studies, some of the more resistance strain of each bacterium selected and were used to test the antibiotic combinations by a chequerboard titration method using 96-well polypropylene micro-titer plates. The factionary inhibitory concentration (FIC) index for combinations of two antimicrobials was calculated according to the equation: FIC index = FICA + FICB = A/MICA + B/MICB, where A and B are the MICs of drug A and drug B in the combination, MICA and MICB are the MICs of drug A and drug B alone, and FICA and FICB are the FICs of drug A and drug B. The FIC indexes were interpreted as follows: <0.5, synergy; >0.5-1, Additional; 1–4, no interaction and >4.0, antagonism. Experiments were performed in triplicate. FIC values are presented as the median of each triplicate.

Results: Combination studies demonstrated synergy and additional effect when use of CM11 peptide in combination of most conventional antibiotics used in this study against all of strains and just Ampicillin and cefotaxime against E.coli, Ampicillin against A. baumannii and Ampicillin and ciprofloxacin against K. pneumoniae have no interaction when used in combine with CM11 peptide.

Conclusion: This study showed that using CM11 peptide with conventional antibiotics as synergism interaction could be enhanced antibacterial activity peptide and antibiotics in a lower MIC. For peptide, decrease of MIC can lead to reduction of its cytotoxicity effect on eukaryotic cells. Also, using peptide in combination with selective antibiotics against antibiotic resistance strain of bacteria, E.coli, A. baumannii and K. pneumonia, can be reduced MIC of these antibiotics to a quarter. It is possible that in synergic interaction model, combination of peptide with antibiotic increased access of antibiotic to the intracellular targets and reduce their MICs. According to our data we suggest that CM11 in combination with conventional antibiotics may be a promising agent for the management of multi drug resistance E.coli, A. baumannii and K. pneumoniae infections.

Keywords: Antimicrobial peptide, E.coli, A. baumannii, K. pneumonia, Synergism, Checkerboard method

P136: **"Introduce of a Simple, Effective and Inexpensive method for DNA Extraction of Malaria parasites from fixed Giemsa-Stained blood slides"**

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Background and Aim: Different factors should be present for having good PCR results, among them the quality and quantity of template DNA has more importance. The blood is the commonest biological sample especially for malaria diagnosis. Extraction of DNA from fixed Giemsa – stained blood smears (Gsbs) is a considerable promising in several aspects of malaria molecular researches. A retrospective epidemiological study for diagnosis of malaria upon the parasites DNA which extracted from positive reported Gsbs was conducted in a hypo endemic area in Iran.

Methods: This study was carried out on the 50 positive fixed Giemsa-stained slides were taken from patients in malarious regions of south and south eastern of Iran across the period 2011-2012. This research perform a nested PCR protocol using new and modified method of DNA extraction from Giemsa – stained blood smears.

Results: Overall 50 samples were analyzed, out of them 36 (72%) had a positive result indicating a high false positive in microscopy method.

Conclusion: In contrast to results of the present study, using DNA extracted from thick or thin smears has produced good results by PCR. This study demonstrated that DNA might be successfully isolated from thin or thick blood smear, indicating that this method of DNA preservation could be considered adequate and convenient for epidemiological studies.

Keywords: Malaria; Plasmodium falciparum; Plasmodium vivax; DNA Extraction

P137: **Urinary tract infection due to GBS in men**

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Background and Aim: Although Group B Streptococcus (GBS) causes sepsis and meningitis in neonates and young infants, but it causes other serious infections with significant morbidity and mortality in pregnant , nonpregnant and men. such as bacteremia ,cellulitis, urogenital infection,wound with specially medical conditions .GBS is transmitted vertically to the newborn during labor and delivery. Among nonpregnant adults, transmission occurs via direct contact; some studies suggest that sexual transmission occurs. Although the incidence and clinical manifestations of invasive disease are well described, little is known about the GBS colonization in men. the incidence of early-onset GBS disease in neonates is decreasing because of the widespread use of intrapartum antibiotic prophylaxis but the incidence of GBS disease in other populations has remained relatively constant. The aim of this study were to evaluate prevalence of urogenital infection in men.

Methods: A total of 6200 sample were enrolled in this study between june 2012 and jun 2013.urine were inoculated on blood agar, chocolate agar, mackanki agar. GBS identified by abillity to produced B hemolytic colonies ,resistance to bacitracin disc , positive camp test , and hydrolysis of sodium hippurate. Anti microbial susceptibility test carry out by CLSI method with penicillin, ampicillin, nitrofurantoin, cephalosporin, ciprofloxacin, tetracycline, urethra (erythromycin and clindamycin)

Results: From 6200 sample 100 (1.6%) GBS isolated 5 isolate were from men (5%). All isolate were susceptibilt to ampicillin, penicillin, nitrofurantoin, cephalosporin, but resistance to tetracycline (80%), erythromycin (38%), clindamycin (51%).

Conclusion: GBS has been transmited via person to person.Several patients in this study with multiple symptoms of UTI and positive UA findings had GBS bacteriuria of between 107 and 108CFU/liter.urinary tract abnormalities choronic renal failure diabetes mellitus and corticosteroid use are among risk factor previously associated withGBS UTI.Although the UTI in men has low incidence but with increased by age men will be at risk to UTI. In one study by Sandra J and et al in 2001 from 289 patient (147 women and 142 men) GBS was isolated from 28 women and 21 men. In our study the age rate were from 45-65. And 5 person GBS in this age group indicate the significant of GBS UTI in elderly men with or without outline disease.

Keywords: UTI, GBS,men

P138: **Seroepidemiology of toxoplasmosis in pregnant women referring to health centers in the city of Hamadan in 2012**

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Background and Aim: Toxoplasmosis is a most common parasitic infection in humans and animals, it has two acute and chronic phases that related to IgM and IgG, respectively. This prevalence is affected by different variables, so determination of the prevalence of serum IgG and IgM antibodies against Toxoplasma in terms of these variables like age,occupation and education level is so important.

Methods: This descriptive-analytic cross sectional study was done on 350 pregnant women referred to health - Therapeutic centers of Hamadan city. After obtaining informed consent from volunteers, their serum samples were tested by IgG ELISA and IgM ELISA methods and their associations with age, occupation and education level variables were measured.

Results: From total of 350 pregnant women, 105 cases (%30) had anti-Toxoplasma IgG, and 3 cases (%2.9) had anti-Toxoplasma IgM. Antibody titer of IgM with variable age, occupation and education level was not significant, but antibody titer of IgG with these variables was significant.

Conclusion: Given the significant association between the disease and age, occupation and education level in pregnant women, it should be provide the necessary training and knowledge about preventiion and avoid of being infected with toxoplasmosis infection.

Keywords: IgG , IgM , pregnant women, toxoplasmosis, Iran.

P139: **Molcular Surveiy of different classes integron amang Pseudomonas**

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Background and Aim: Antibiotic resistance in Pseudomonas aeruginosa one of an important pathogen commonly implicated in nosocomial infections has had increased in recent years and the presence of integrons and associated resistance gene cassettesis well established. Rapid acquisition multi drug resistance has led to high mortality in patients with nosocomial infections. The aim of this study is to investigate of classes 1 and 2 and 3 integrons among P. aeruginosa isolated from ICUShahid Beheshti Hospital and determine the sensitivity towards different antibiotics

Methods: This cross - sectional study is done on 54 P. aeruginosa isolated from different places and devices of ICU of Shahid Beheshti Hospital in Babol, north of Iran, during the years 2011-2012. After cultivation, isolation and extraction of DNA, class 1and 2 and 3 integron gene was investigated by PCR. Also for the sensitivity test to antibiotics of Ciprofloxacin, Cefazolin, Ceftriaxone, Ceftizoxime, Cefotaxime, Amikacin, Ofloxacin, Imipenem, Cefepime, Ticarcillin and Gentamicin,Ceftazidim this was performed by micro dilution and disk diffusion methods.

Results: 22out of 54 (40/7%) of P. aeroginosa had intl gene, and1outof 54(1/9) P. aeroginosa had int 2 and class 3 integron .No significant differences were seen between the presence of integron gene and resistance to the antibiotics except to Ofloxacin. The most resistance was observed inCefepime (100%) and the lowest to Ciprofloxacin (38/9 %).

Conclusion: The result of this study showed antibiotic resistance of P. aeruginosa, isolated from the ICU environment and equipment, is very high. It seems that the source of this bacterium comes from a cloned bacterial gene with a high prevalence among hospital strains that may cause transfer resistance to the same or different species

Keywords: Pseudomonas aeruginosa, class 1 integron, Antibiotic Resistant.

P140: **An Investigation on Drug Resistance Among klebsiella pneumoniae Strains Isolated from North of Iran and the Relationship with the Presence of Different Classes of Integron**

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Background and Aim: Klebsiella pneumoniae is an important cause of nosocomial infections. The prevalence of antibiotic resistance is growing among strains of this bacterium. In this research the role of different classes of integron in creating drug resistance in Klebsiella pneumoniae has been studied

Methods: In this descriptive-analytical study, 50 strains of Klebsiella pneumoniae were isolated from the ICU of Shahid Beheshti Hospital, Babol and antibiotic susceptibility test was performed on them through disk diffusion method with 11 antibiotics including cefotaxime (CTX), amikacin (AN), ofloxacin (OFX), imipenem (IPM), cefepime (FEP), ticarcillin (TIC), gentamicin (GM), ciprofloxacin (CP), cefazolin (CZ), ceftriaxone (CRO), and ceftizoxim (CT).

Results: Of 50 isolated strains, 21 strains (42%) contained integron gene; 16 strains (32%) had integron class 1 and 5 strains (10%) had integron class 2. No class 3 integron was found among the strains. Also, none of the strains simultaneously carried class 1 and 2 integron. Resistance to the above antibiotics was 66%, 38%, 50%, 50%, 64%, 62%, 60%, 52%, 88%, 60%, and 88%, respectively. Forty samples (80%) were resistant to multiple antibiotics

Conclusion: The results of this study, showed the high prevalence of integron gene in different strains of Klebsiella pneumoniae, isolated from different parts of the ICU's environment and equipment. Also, the rate of antibiotic resistance was high among studied strains that can be a warning for infection with the bacteria. Based on this study, antibiotics such as amikacin, imipenem, and ofloxacin, with the least amount of resistance, can be effective in the treatment of Klebsiella pneumoniae infections.

Keywords: Klebsiel,la pneumoniae, antibiotic resistance, integron

P141: **Razi Bacillus anthracis Sterne 34F2, domestic or exotic?SNP analysis answers**

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Background and Aim: On his return from a year-long visit of Veterinary Laboratory Agency (VLA) at Weybridge , UK , the late head of Anthrax vaccine department of Razi institute, Dr Iraj Arabi brought back a valuable souvenir. A lyophilized vial of Bacillus anthracis Sterne 34F2 strain He made history in a century of anthrax vaccine production in Iran as a shift from old Pasteur C strain to the safer strain of Sterne34F2 was materialized. Almost half a century on, a question has now raised concerns over potential genomic variations in the original imported strain due to the subsequent sub-cultures at Razi. Recent MLVA genotyping observations are not in support of such variations. In the present work we have described what further information have been achieved by Single Nucleotide Polymorphism analysis in backing genomic stability of the Sterne 34F2 strain we keep today at Razi.

Methods: Using a domestically developed PCR protocol, 13 well-established SNPs from genome of Razi Sterne 34F2 daughter strain were amplified, sequenced and characterized. These were compared against the published SNPs of Strene 34F2 strain from external sources.

Results: All the 13 SNPs from the Razi Sterne 34F2 daughter strain were characterized. No change was detected at any SNPs locus.

Conclusion: Previously, MLVA findings at Razi showed no extensive change in the genomic content of the Sterne 34F2 strain. As in genomics SNPs are typically considered "slower" than Tandem Repeats (TRs) in experiencing variations, our finding that the today's Sterne 34F2 strain at Razi bears no SNPs different to its British ancestor seems not odd. A further intriguing finding of our study that the Sterne 34F2 SNPs pattern is different to those from Iranian B. anthracis isolates is not surprising either as the domestic isolates have evolved locally during the events not related to evolution of the Sterne 34F2 strain outside Iran .

Keywords: Bacillus anthracis,SNP analysis ,Sterne 34F2

P142: **Detection of Pseudomonas aeruginosa by PCR and designed primer pairs of 16S rDNA**

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Background and Aim: Members of Pseudomonas are aerobic Gram negative, chemo-organotrophs bacteria. Species of P. aeruginosa is often isolated from clinical samples and usually identified by conventional biochemical tests. The purpose of this study was to design a specific primer pair of 16S rDNA gene for molecular identification of P. aeruginosa using polymerase chain reaction (PCR).

Methods: In this study, primer pairs of 16S rDNA designed by AlleleID6 software based on the target sequence in FASTA format of an accession number in NCBI site. Primer BLAST performs for checking specificity, when both primers provided. PCR amplification of the 16S rDNA gene was performed with designed primers, 16S-PA-F (5'-CACCTGGACTGATACTGACACTGAG-3') and 16S-PA-R (5'-CCTACGGCTACCTTGTTACGACTTC-3') yielding a 780 bp product. P. aeruginosa ATTC 9027 and E. coli PTTC 1330 were as positive and negative controls, respectively. 60 P. aeruginosa clinical isolates were collected from different clinical specimens and identified by conventional biochemical tests. Total DNA genomic were extracted from pure cultured bacteria using chloroform method by Chen and Kou method. Reaction mixtures were prepared with DNA extractions as will be explained in full paper. PCR products were electrophoresed in 1.5% agarose gel and stained with ethidium bromide. A 100 bp DNA ladder (Fermentas, Lithuania) was used as molecular weight marker.

Results: After setting PCR technique, DNA amplification program was designed as an initial denaturation step (95?C, 5 min) followed by 30 cycles of denaturation (96?C, 30s), annealing (56?C, 30s) and extension (72C, 6?0s) and a single final extension of 5 min in 72?C. PCR detecting 16S rDNA gene was positive in 60 (100%) clinical isolates. Results of PCR for control strains of P. aeruginosa and E. coli were positive and negative, respectively.

Conclusion: We have developed a rapid, reliable and sensitive method to analyze and detect of P. aeruginosa using 16Sr DNA. This PCR assay was found to provide highly specific detection and could be successfully used to identify P. aeruginosa in different clinical isolates.

Keywords: Pseudomonas aeruginosa, 16S rDNA primer, Identification, PCR

P143: **A One year Epidemiological study of Superficial cutaneous in Suspicious Patients in Tehran(2011-2012)**

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Background and Aim: Identification of different species of dermatophytes and cutaneous mycosis agents for finding infection sources and also educating the community in order to familiarize with consequences of contacting infected people or animals will be helpful; and on this basis, the main objective of this study has been determination of distribution and dissemination ways of superficial fungal skin diseases.

Methods: Records of 850 patients suspected to cutaneous mycosis were evaluated for presence of fungal infection in a one-year time period from October 2011to October 2012. Skin specimens were collected from patients through skin scraping. Diagnoses were based on direct microscopy examination and culture in accordance with routine laboratory methods of mycology

Results: In total, 350 cases (41.17%) were inflicted with cutaneous mycosis, of which, dermatophytosis, with 170 cases (48.6%) was the most prevalent disease of this type. The remaining included 46 cases (13.1%) of tinea versicolor, 43 cases (12.3%) of erythrasma, 51 cases (14.6%) dermal candidiasis and 40 cases (11.4%) of fungal infections of nails caused from saprophyte moulds. The most common clinical form was tinea pedis with 53 cases (31.2%). Among dermatophytes isolated from cultures, Tricophyton mentagrophytis was the most prevalent agent with 41 cases (40.2%).

Conclusion: This study shows that dermatophytosis is still an important fungal skin disease among cutaneous mycosis.

Keywords: Dermatophytosis, Tinea Versicolor, Candidiasis

P144: **Frequency study of cagA among Helicobacter pylori isolates from patients with gasteroduodenal disorder attending to Imam Reza Hospital in Kermanshah (2011-2012).**

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Background and Aim: Helicobacter pylori is the causative agent of gastritis, peptic and duodenum ulcer, gastric adenocadcinoma and mucosal associated lymphoid tissue (MALT). More than half of the world's population is infected by this genus. Probably the most important virulence factors of Helicobacter pylori is cagA, which may involved in inflammation, progression and retention of the disorders.The purpose of this study was to investigate the prevalence of cagA among Helicobacter pylori isolated from gastric disorder patients referring to Imam Reza hospital in Kermanshah.

Methods: Two biopsy specimens were collected from patients with gastrointestinal disorders. One sample was cultured on Columbia agar medium containing egg and urease test were carried out on another sample. After DNA extraction and confirmation of Helicobacter pylori using amplification of 16SrRNA gene, specific primers were employed to evaluate the presence of cagA gene. Furthermore, lack of cagA gene was confirmed based on applying Cag PAI empty site.

Results: From 96 investigated samples, 76 patients sufferer from gastritis, 20 cases had gastric and duodenal ulcer. Among the isolates, 81 (84%) carried cagA, and cagA prevalence were 84.2%, 84 .61% and 85.7% in gastritis, gastric ulcer and duodenum ulcer, respectively. Statistical tests showed that there is no significant relationship the cagA gene and the disease.

Conclusion: According to this study, it is assumed that cagA+ strains have no role in the pathogenesis of the disorders but may increase the progression of peptic ulcer.

Keywords: Virulence factors, Cag PAI empty site, CagA, Helicobacter pylori.

P145: **the effect of aqueous extract of Elaeagnus angustifolia on serum IL-6 level in mice were received Brucella abortus LPS**

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Background and Aim: the incidence of inflammatory diseases is increasing worldwide . current Therapies are not provide a substantial improvement for patients . recent studies have found that Elaeagnus angustifolia fruit extract has anti-inflammatory effects . conversely the IL-6 is elevated in several inflammatory diseases and thought to be a byproduct of inflammatory responses and a marker of inflammation . We decided to study the effect of Elaeagnus angustifolia on serum level of mice that were received Brucella abortus LPS .

Methods: souri mice were evaluated through the injection of different doses (10 ، 20 ، 30 and 40 mg/kg) of extract to the peritoneum in four days . in fourth day of each mouse was injected peritonealy with 100 microgram Brucella abortus LPS . Blood was collected from heart in next day and serum was separated and stored at -80℃ until assay Serum IL-6 levels were measured by Elisa kit .

Results: the research data showed significant decrease serum IL-6 level in all treatment groups Compared to control .

Conclusion: one of the anti-inflammatory mechanism of Elaeagnus angustifolia fruit extract is Decrease serum IL-6 level .

Keywords: LPS: Lipopolysaccharide , IL-6: intrleukin-6

P146: **Identification of casual infection agents in acute otitis media of children referred to Shiraz Khalili hospital (Shiraz-IRAN)**

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Background and Aim: Acute otitis media (AOM) is one of the most common diseases in pediatrics age. Based on prospective studies, >75% of children experience this disease at least once by the age of 3 years and about 50% of persons will suffer for otitis at least three times over the course of the life's. Globally, the major bacterial pathogens responsible for AOM are Streptococcus pneumoniae, Haemophilus influenzae, Moraxella catarrhalis, Streptococcus pyogenes, Staphylococcus aureus, Helycobacter pylori and recently introduced Pseudomonads, beside these bacteria, Viruses are another agent for this disease which alone are found in approximately 20% of cases, while co-infection with bacteria occurs in two thirds of cases. According to the importance of identification of casual agent of AOM for prepare treatment in pediatrics age in this study we tried to evaluate the different probable bacterial and viral agents may results for this disease.

Methods: In a prospective study between 2010-2011, in khalili hospital (Shiraz-IRAN), 41 middle ear fluid samples from 21 pediatrics (age ≤19) with Ear, Nose and Throat Specialist –confirmed AOM, was obtained by tympanocentesis. All samples were extracted for total DNA by extraction kit. Extracted DNA's were tested for introduced bacteria and viruses agents with molecular PCR method with specific primers for each one separately. The PCR products were analyzed by electrophoresis.

Results: In 41 bilateral ear effusion samples, all of the samples were negative for all bacterial and viral infectious agents except 14 (33%) of samples which were positive for pseudomonas

Conclusion: Results of our study showed that unlike other studies in our center Pseudomonas is more conquer infectious agent. According to the importance of this bacteria in pathogenicity, it highly recommended to consider this agent for AOM to be treated

Keywords: Acute otitis media, pediatrics, Bacteria, Viruses

P147: **Antibacterial effects of Juniperus communis leaves extract on gram positive and gram negative bacteria**

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Background and Aim: Gram positive and gram negative bacteria are examples of the most important pathogenic agents affecting human health. Considering the bacteria's increasing resistance against chemical drugs, it becomes essential to discover compounds without side effects which are made of from medicinal plants. The objective of this study is to survey the effects of aquatic extract of Juniperus communis leaves on Staphylococcus aureus (gram positive), Escherichia coli (gram negative) and Salmonella typhimurium (gram negative) bacteria.

Methods: J. communis leaves are collected from Arasbaran in East Azerbaijan and after bening confirmed by botanical specialist, leaves have been dried. Their aquatic extract is prepared using the perculation method. Extracts anti bacterial effect on bacteria which were obtained from Persian Type Culture Collection, was measured using well diffusion method in Mueller hinton agar media. Each test was repeated tree times, average diameter of inhibitory zone was measured and noted, afterwards, using macro dilution method in broth, minimum inhibitory concentration rate was determined.

Results: According to the results of antimicrobial activity tests, J. communis leaves extract is most effective on E.coli and least effective on S.typhimurium.

Conclusion: Taking account of test results, J. communis leaves extract's has a significant antibacterial effect on E.coli. More in vitro research is needed to find out this extract potential to treat bacterial infections.

Keywords: Anti bacterial, Juniperus communis, Arasbaran, leaves extract, E.coli, East Azerbaijan

P148: **The over expression of spoT gene in coccoid forms of Helicobacter pylori**

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Background and Aim: Helicobacter pylori (H. pylori) can infect into the gastric epithelial cell to cause benign or malignant disorders. Under stressful environment, a spiral form of H. pylori is altered into a nonculturable, but viable coccoid form. The coccoid forms may be complicated in transmission of infection and in relapses subsequent antimicrobial therapy. H. pylori must adapt to fluctuating conditions in the harsh environment with the use of a minimal number of transcriptional regulators. The aim of this study was to investigate whether H. pylori utilizes the stringent response, relating signaling through the alarmone (p)ppGpp encoded by spoT gene, as a survival strategy during antibiotic stresses.

Methods: The spiral forms of standard ATCC 26695 H. pylori strain and 9 clinical isolates were cultured under 1/2 MIC concentrations of amoxicillin for induction of coccoid forms. The viability of induced coccoids was evaluated by flow cytometry method and the quantitative mRNA expression level of spoT gene was megueared and compared in spiral and coccoid forms of H. pylori by quantitative real-time PCR method. We analyzed data using SPSS 20 and independent T-test.

Results: According to our flow cytometry analysis, the sub MIC (1/2 MIC) concentration of amoxicillin induced the viable coccoids (>80%). Quantitative RT real-time PCR showed that the expression of spoT gene in induced coccoid forms was significantly ($p< 0.0001$) higher than in spirals (30 fold). This is the first report of quantitative spoT expression level in antibiotic induced coccoid form compared with untreated spiral form of H. pylori.

Conclusion: H. pylori is capable to transfer to spore-like stage VBNC coccoid form under the sub MIC concentration of antibiotics especially those that act on bacterial cell wall such as amoxicillin. Meanwhile it provides an explanation of why the recurrence of infection occurs after the treatment. The dormant coccoid form is resistant to antibiotics. We conclude that the silent and persistent coccoids appear to be transcriptionally active and modify their transcription to adapt to environmental conditions. Based on the transcription profiles, the over expression of the spoT regulatory gene in coccoids may be contribute to the adaptation, resistance and persistency of coccoids. SpoT acting as a global transcriptional regulator influences transcription of genes related to important metabolic pathways, thus affecting various bacterial physiological processes including resistance to antibiotics.

Keywords: Helicobacter pylori, coccoid, spoT, gene

P149: **Survey of turanose metabolism in methicillin sensitive and resistant staphylococcus aureus Strains**

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Background and Aim: Methicillin resistant Staphylococcos aureus (MRSA) strains have a modified form of a PBP2 protein called PBP2a which is coded with mecA gene. The purpose of this study is to apply turanose fermentation phenothypic method to identify these isolates.

Methods: Out of 150 Staphylococcos aureus isolates, 40 sensitive and 40 resistant strains to methicillin were collected. Following the Biochemical tests, MRSA strains were identified by oxacillin 1 µg disc. Molecular identification of mecA gene was carried out by PCR. Sensitive and resistant strains of Staphylococcos aureus were exposed to different dilutions (1%, 0.7%, 0.5%) of turanose using microplate technique.

Results: The mecA gene was identified in all of the MRSA isolates by PCR method. The significance difference was observed at turanose 0.7% between sensitive and resistant isolates because of the absorbance rate of all MRSA strains at 610 nm wavelength were higher than sensitive strains.

Conclusion: Resistance to methicillin in Staphylococcos aureus is usually in accordance with the ability of carbohydrate metabolism. This ability in MRSA isolates is may be related to facilitated transformation of the carbohydrate in presence of PBP2a. In present study turanose metabolism on 0.7% was more in MRSA than sensitive strains. Because rapid identification of MRSA isolates are very important in nosocomial infections, usage of rapid phenotypic methods are cost effective and accurate.

Keywords: Staphylococcos aureus , Turanose, mecA gene.

P150: **Epitope Prediction for Different Protein of Human Papillomavirus (HPV) as Initial Step for Production of Universal Vaccine**

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Background and Aim: Cervical cancer is the second most common cancer among women worldwide, and 93 percent of invasive cervical cancers worldwide caused by human papillomavirus (HPV). Of the >200 HPV genotypes, six 'high-risk' types of HPV (16, 18,31, 45, 52 and 58) are of particular importance, because they have been highly associated with over 80% of all cervical cancers. Many studies have found continued expression of E6 and E7 proteins in the majority of cervical cancer cases, but not in normal tissues. T-cells are the most important effector cells for therapeutic vaccination strategies. For this reasons, we defined project in Pasteur institute of Iran for production global vaccine for HPV. So, Aim of this study is to presents of our first step results for creating a successful vaccine.

Methods: At first HPV protein sequences of high-risk serotypes (for E6 and E7) retrieved from NCBI databank. After alignment, different E6 and E7 sequences by hybrid prediction method tested for epitopes that have immunogenicity for T cell and potency to attachment to HLA-A*02: 01 (A2) and H2-Kd. Selected epitopes attached to each other in different patterns and evaluated for best statuses of proteasome cleavage sites. Also Codon optimization was done for compatibility of designed vaccine with different expression systems.

Results: Result of Hybrid method and also selection among 6 high risk serotype sequences that were associated with HLA-A*02: 01 allele; HPV16 E6 29-38 (TIHDIILECV) , E7 11-19 (YMLDLQPET), HPV18 E6 24-33 (SLQDEIEITCV), E7 88-97 (QLFLNTLSF); HPV31 E6 90-98 (FLNTLSFVC), E7 81-90 (ELLMGSFGLV); HPV45 E6 24-33 (SLQDVSTACV), E7 89-98 (QLFLSTLSFV), HPV52 E6 45-53 (FLFTDLRIV) E7 84-92 (MLLGTLQVV); HPV 58 E6 45-53 (FVFADLRIV) E7 83-91 (LLMGTCTIV), and associated with mouse H2-Kd allele; Hpv16 E6 98-107 (QYNKPLCDLL), E7 80-89 (EDLLMGTLGI); HPV18 E6 33-41 (VYCKTVLEL), E7 85-94 (AFQQLFLNTL); HPV31 E6 44-53 (DFAFTDLTIV), E7 56-65 (TFCCQCKSTL); HPV45 E6 89-98 (TLEKITNTEL), E7 86-95 (TLQQLFLSTL). At least 8 fusion peptide that had best patterns of proteasome cleavage sites in construct choose.

Conclusion: For more accuracy of processes of prediction and designing, we used different methods (hybrid approach) to predicts T cell epitopes. Polytopic vaccine (based on in silico approaches) causes concentration and increasing immune responses to important epitopes and decrease adverse effects of vaccination. This study is first step on designing a Universal vaccine for HPV based on CTL epitopes for effectively control of HPV infection and development cervical cancer therapeutic regime.

Keywords: human papillomavirus (HPV), T cell, Epitope prediction, Rational vaccine

P151: **Detection of metallo-beta-lactamases (MBL) producing**

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Background and Aim: Pseudomonas aeruginosa is a causative agent of nosocomial infections. Metallo-beta-lactamases (MBLs) are one of the agents that cause resistance of Pseudomonas aeruginosa to carbapenem. Because of important role of carbapenemes in treatment of Pseudomonas infections, so in this study, the metallo-beta-lactamases producing Pseudomonas aeruginosa were investigated.

Methods: A total of 236 Pseudomonas aeruginosa isolates were collected from hospitalized patients clinical specimens in Imam Khomeini and Golestan hospitals of Ahvaz, Iran. After identification of the isolates by standard biochemical tests, their antimicrobial resistant patterns to 10 common antibiotics were determined based on CLSI protocol by disk diffusion agar method. Finally, the metallo-beta-lactamases production by Pseudomonas aeruginosa isolates was identified by Imipenem-EDTA combined disk method.

Results: Among the 236 Pseudomonas aeruginosa isolates , overall 122 isolates (51.4%) were resistance to imipenem . Based on the results of Imipenem-EDTA combined disk method, production of metallo-beta-lactamases were known in 110/122 (90%) Pseudomonas aeruginosa isolates.

Conclusion:: Since MBLs producing Pseudomonas aeruginosa are resistance to all other betalactam antibiotics, it is crucial to be screened imipenem non-susceptible isolates of Pseudomonas aeruginosa for MBLs production.

Keywords:: Pseudomon asaeruginosa, Imipenem-EDTA combined disk method, Metallo-beta-lactamase.

P152: **Association of Serum Levels of High-Sensitive C - reactive protein (hs-CRP) with activity inflammation in patient of chronic gastritis**

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Background and Aim: Gastritis one of the world's most important premalignant lesions. Recent studies suggest the production of inflammatory cytokine-like C-reactive protein in gastritis. The purpose of this study is to determine the relationship between hs-CRP and activity inflammations in patients with chronic gastritis

Methods: two hundred thirty nine patients with dyspepsia were included in this study. Demographic and clinical variables and chronic medication used by The Sydney system in this system have a histopathologic finding include H.pylori staining of gastric mucus and serum concentration of hs-CRP by use neflumetric assay was measured. Independent correlation between hs-CRP and activity inflammation was tested by logistic regression analysis.

Results: In total 221 patients (56.6% female) with mean age of 40 years (range between 10 to 90years) entered the study.The prevalence of mild, moderate, severe activity inflammation respectively was 66.5%, 23.8%, 9.6%.Mean and SD hs-CRP levels in men was 2.85 ±2.84 mg /dl and in women is 2.80±4.80 mg / dl (p =0.047). SD Mean hs-CRP in patients with chronic inflammation is 2.73±3.750mg/dl, in patients with active inflammation is 2.83±3.80mg/dl, and patients with Helicobacter pylori infection, gland atrophy, metaplasia and dysplasia, respectively, 3.52 ± 5.05mg/dl , 2.22 ± 2.38 mg / dl and 5.28 ± 5.05 mg / dl, respectively Between hs-CRP and inflammation was a significant relationship(P = 0/011),so with increasing the severity of active inflammation the higher average hs-CRP will seen .Sensitivity of logic link function model in patients without active inflammation or mild, moderate and severe active inflammation is 95.4%, 72.1% and 88.9% respectively.

Conclusion: Although serum hs-CRP is not a specific biomarker for gastritis, Elevated hs-CRP levels may be predictive of active changes by gastric mucosa and a promising therapeutic target for patients.

Keywords: hs-CRP, Chronic Gastritis, activity inflammation, helicobacter pylori

P153: **Comparison between the effectiveness of Furazolidone and Clarithromycin on eradication of helicobacter pylori among patients with peptic ulcer**

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Background and Aim: Helicobacter pylori infections occur in about 50% of the world population and have a main role in the creation of peptic ulcer and its related cancer. Immethodical use of antibiotics, causing antibiotic resistance, induces many problems in the means of eradication of this infectious agent. This study aims to evaluate the effectiveness of two treatment regimens on the eradication of helicobacter pylori and its complications among patients with peptic ulcer attending to a gastroentrology clinic in Ilam.

Methods: By a clinical trial, patients with peptic ulcer showing an infection compliance of more than 80%, confirmed by biopsy, were entered into the study and were randomly divided in two groups. Group A was treated by medical regimen of Amoxicilline, Bismot, Omeperasol and Furazolidone (ABOF) and group B by medical regimen of Amoxicilline, Bismot, Omeperasol and Clarithromycin (ABOC) for two weeks. In the next step, both groups were treated by only Omeperasol for two weeks and finally, the infection rate of helicobacter pylori was investigated by urea breath testing among both groups.

Results: Among the 137 registered patients, 17 were unable to continue the treatments and the associated follow ups. The mean age of patients in group A, including 30 male and 30 female, was 36.1 years and for group B, including 23 male and 37 female, was 40.1 years. The rate of infection eradication (negative urea breath test) in group A was 38.3% (23 patients) and in group B was 53.3% (32 patients) without a significant difference (P= 0.07). The most common symptoms among patients in group A were a bad taste mouth (80%), headache (70%) and abdominal pain (67%) and in the group B including headache (62%), a bad taste mouth (60%) and abdominal pain (58%), respectively. Generally, there was no significant difference for symptoms between two treatment regimens except for the bad taste mouth (P= 0.01).

Conclusion: Although the current study did not show a significant difference between the two medical regimens of ABOF and ABOC, but the ABOF compared to ABOC, showed a lower rate of infection eradication as well as higher complications for patients. According to these results, the ABOC was considered as the preferred regimen. Urea breath test is suggested to be applied for all patients under treatment for helicobacter pylori infections.

Keywords: Peptic ulcer disease, helicobacter pylori, eradication, furazolidone, clarithromycin

P154: **Victory model and theory; Simulation of structures and functions of Antibody and MHC by innovative hands, forehand and arms modeling techniques**

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Background and Aim: Hand, forehand and arms (forelimb) molecular modeling for showing of Antibody and MHC structures and functions named "Victory model" in this article, that encompasses immunoanatomical models used to mimic the behaviour of biological molecules. "Victory model" name come from "Y"-shaped of antibody when we showing it by forelimb.

Methods: The study consists of the three major phases: (a) conceptualization of the model based on the literature reviews in field of immunology, biochemistry, anatomy and computer science, (b) visualization the models by forelimb, and (c) refinement of the models and checking their quality of displaying concepts.

Results: About 72 different models obtained for showing different antibody structures and Isotypes (IgM, IgG and etc.), Ig variable and constant regions, disulfide bonds, Immunoglobulin diversity, Somatic hypermutation and affinity maturation, Activation of complement and interaction with other molecules and cells. Also, 25 forelimb models obtained for MHC class I and II structure and functions. In this study some hidden aspects of antibody interactions and functions, cause touchability of models with eyes, were defined that never have been understood.

Conclusion: The findings of the study presents a models for designing innovative training projects that enable teachers to better transfer concepts to student in immunology classes and students can simply exercise and have deep, accurate and touchable insight to structure and functions of antibody and MHC. Besides, it may raising discussions that forelimb may be takes some evolutions patterns and shaping forms from specific antigen recognizing proteins as both have responsibility in Control of Objects.

Keywords: Antibody, MHC, hands, forehand, arms, modeling

P155: **Extended spectrum beta lactamase producing Acinetobacter baumannii isolated from Tehran, Iran**

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Background and Aim: Acinetobacter baumannii produce drug resistant infections.Production of extended-spectrum β-lactamases (ESBL) is one of the main resistance mechanisms against new cephalosporins in A. baumannii. Therefore, surveillance of antibiotic resistance pattern of A. baumannii is necessary for infection control programs. This study was carried out to determine the prevalence of β-lactamase genes of bla TEM A. baumannii in Tehran, Iran.

Methods: In total, 75 A. baumannii isolates, isolated from a tertiary care hospital in Tehran, Iran. The resistance to ceftazidim was assessed by Kirby-Bauer disk diffusion method. The confirmatory test for detection of resistant isolates was carried out by double disk method at the presence of ceftazidim and clavulanic acid. The presence of β-lactamase gene of bla TEM , in ESBL was assessed by PCR.

Results: Among eht 75 A. baumannii isolates, 70 isolates (93.3%) are resistant to ceftazidim. Eight (10.7%) of them are confirmed as ESBL producing A. baumannii. ?-lactamase genes of bla TEM by the product size 925 bp can be seen in 10 (13.3%), isolates.

Conclusion: Multi–drug resistant A.baumannii isolates are rapidly spreading in our patients. The prevalence of β-lactamase genes of bla TEM A. baumannii in Tehran is in the range of country average.

Keywords: A.baumannii, ESBL, bla TEM , PCR

P156: **Prevalence of ISampC gene among Acinetobacter baumannii isolates and its correlation with resistance to ceftazidime**

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Background and Aim: Resistance to β-lactams is related mostly to the expression of β- lactamases, whereas alterations of outer-membrane permeability, modification of penicillin-binding proteins and increased activity of efflux pumps play a secondary role. While A. baumannii naturally produces an AmpC-type cephalosporinase, which is expressed normally at a basal level, it does not reduce the efficacy of expanded-spectrum cephalosporins (ESCs) or carbapenems. The addition of specific insertion-sequence (IS) elements ISAba1 (belonging to the IS4 family) upstream of the blaampC gene enhances the expression of this AmpC β-lactamase by providing promoter sequences, resulting in resistance to ESCs (ceftazidim).

Methods: Samples were processed with routine laboratory methods and gram- negative bacilli were identified by standard tests. The resistance to ceftazidim was assessed by Kirby-Bauer disk diffusion method. ISAmpC were screened by PCR.

Results: A total of 75 consecutive nonduplicate isolates of Acinetobacter baumannii were collected by standard tests. yeneveS (93.3%) isolates of A. baumannii were resistance to ceftazidim. The presence of insertion sequence upstream of ampC (ISAmpC) genes by the product size 1700 bp was confirmed by PCR in 64% (n=48) fo A. baumannii clinical isolates.

Conclusion: Presence of an insertion sequence upstream of AmpC in A. baumannii clinical isolates has the potential to cause over-expression of AmpC, resulting in high-level ceftazidime resistance.

Keywords: A. baumannii, ISAmpC, ceftazidime

P157: **Molecular analysis and susceptibility pattern of MRSA strains in emergency department patients and related risk factors**

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Background and Aim: Methicillin -resistant staphylococcus aureus (MRSA) have become widespread in hospital and community and Staphylococcus aureus nasal colonization has been identified as a risk factor for MRSA infections. This study was conducted to determine the prevalence of MRSA nasal colonization among adult patients in emergency department(ED) by considering the related risk factors and antibiotic susceptibility patterns.

Methods: A cross-sectional study was conducted in 810 patients referred to an ED in Kashan,Iran. Anterior nares cultures were obtained and a questionnaire including the risk factors for MRSA colonization was filled for each patient. A multiplex PCR assay was employed for SCCmec typing. For detecting Panton Valentine Leukocidin(PVL) genes, a PCR assay was used. The susceptibility of MRSA isolates to amikacin, clindamycin, gentamicin, ciprofloxacin, SXT, erythromycin, tetracycline, penicillin, vancomycin and cefoxitin were determined by using disk diffusion method.

Results: Two hundred and ninety six (36.5%) and 26(3.2%) out of 810 patients were S.aureus and MRSA nasal carrier, respectively. 9(34.6%), 7(26.9%), 2(7.7%), 2(7.7%), 2(7.7%), 1(3.8%), of MRSA isolates were classified as type V,III, I, IV b, IV h, IV a respectively and 7(26.9%)of them were nontypeable. PVL genes were not detected. All of MRSA isolates were multi drug resistant (MDR).

Conclusion: Statistically significant correlation between previous hospitalization, usage of urine and vein catheters and MRSA colonization was observed. Having more information of the epidemiology and risk factors for nasal MRSA colonization can be helpful for the treatment and prevention of MRSA infections.

Keywords: Molecular analysis, susceptibility pattern, MRSA, emergency department, risk factors

P158: **Isolation of Biofilm producing Pseudomonas aeruginosa from patients in burn unit and the comparison with other isolated P. aeruginosa from patients along with the effect of hypochlorite disinfectant**

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Background and Aim: Pseudomonas aeruginosa grow in the hospital environment and are attached to surfaces and develop biofilm. Biofilms are considered as a pathogenic source in hospitals and thus are able to remain for a longer period of time. The presence of biofilm inables antimicrobial and biocides agents and in result the Pseudomonas aeruginosa bacteria is protected from elimination of the common disinfectant methods. Chemical disinfectants such as sodium hypochlorite are usually used as surface disinfections in hospitals thus this chemical agent was evaluated for its rule in biofilm inhibition.

Methods: Identification of Pseudomonas aeruginosa was done by standard biochemical tests bacterial susceptibility to antimicrobial agents was determined by disk diffusion method. Detection of biofilm was done by microtiter-plate screening method. Variated concentrations of sodium hypochlorite were used as an inhibition of biofilm formation.

Results: Among the experimented bacteria 26.7% formed a moderate biofilm and only one case 0.7% formed a strong biofilm also in 109 cases which was 72.6% were unable to form a biofilm. Our data show that in all concentrations hypochlorite is able to inhibit biofilm formation.

Conclusion:: Our data suggest that sodium hypochlorite is an appropriate disinfection agent against biofilm formation in Pseudomonas aeruginosa

Keywords: Pseudomonas aeruginosa, Biofilm, Sodium hypochlorite

P159: **Distribution Frequency of Mycoplasma hominis in Endocervical Specimens of Patients Referred to Qazvin Kowsar University Hospital by Culture and real-time PCR**

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Background and Aim: It was thought that Mycoplasma hominis is a commensal bacterium in women's genital tract, but in recent years the presence of this organism in human body is considered as a potentially pathogen. In this study we applied culture and real-time PCR techniques for detection of Mycoplasma hominis in endocervical specimens of patients referred to Qazvin Kowsar University Hospital in 2012. In this research we have also evaluated the relationship between presence of this organism and appearing disorders such as vaginosis, infertility, abortion and preterm labor.

Methods: In this research, 226 patients with at least one of the mentioned disturbances were studied and endocervical swabs were collected from each of them. Culture technique by eliminating the filtration step and real-time PCR targeting 16s rRNA was applied as two diagnosis methods.

Results: Frequency of Mycoplasma hominis in this studied population was 15.5 percent by both methods. Association of this organism with some disorders was significant so that 28.5% of patients with infertility and 17% of patients with vaginosis were colonized by Mycoplasma hominis.

Conclusion: Frequency of Mycoplasma hominis in this studied population is considerable and more investigations are necessary. Although molecular based techniques such as real-time PCR are high sensitive, culture method is still valuable for detection of M. hominis.

Keywords: mycoplasma hominis, infertility, abortion, vaginosis, PPLO

P160: **Molecular detection of beta-lactamase genes (blatem, blactx) drug resistant and Glutaraldehyde in isolates of Acinetobacter baumannii separated from surfaces and equipments in selected hospitals of Te**

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Background and Aim: Unstoppable consumption of antibiotics and disinfectants in hospital environment and society declares as one of the most important coding factors that cause appearance, evaluation and gaining new resistance of bacteria across from anti bacterial materials. These factors have provided the conditions of creating clones and bacteria isolates specially Acinetobacters kind with having Phenotypic and Genotypic different features between the pathogens of non-fermented that have achieved PDR, MDR and XDR mechanisms.

Methods: In this time-defining 131 Acintobacter baumannii out of 588 sent samples from surfaces and medical equipments related to special parts of the different hospitals of Tehran that sent to microbiology lab of medical university from 1391/09/01 till 1391/11/30 and identified with biochemical tests. Isolates considered from the point of resistance and sensitivity against 12 antibiotics (taken from the company MAST DIAGNOSTICS) with disk diffusion method and with minority of the inhibitory density with Microbroth dilution method against Imipenem and Meropenem Antibiotics according to clinical and laboratory CLSI criteria. Meanwhile the strains of Acintobacter baumannii (ATCC = 19606) was used as control strains.

Results: In this research the resistance percentage of isolates against imipenem and meropenem and 131 linkomicin isolates (100%) and 117 ceftozoxim and ecsi cilin isolates(90%) and 112 gentamicin isolates (85/97%) and 121 ciprofloxasin isolates (98%) and 129 ceftazidim isolates (99%) and all isolates were sensitive against kelestin. And the minority of inhibitory density against imipenem and meropenem in 79 isolates (60/31%) was more than 64 micrograms out of milliliter. The frequency percentage of blactx genes in resistant isolates was 61/2% and there was no resistance from resistant isolates having blactx genes against glotaroied 2%.

Conclusion: In this research the appearance importance of Acintobacter baumannii considered and because the mechanism of the resistance against isolates antibiotics mediated with genes wich are on genetics elements as like as trans pozon and integron and plasmids and cromozoms. And if resistant isolates that has blactx genes and be resistant to glotarid 2%, their resistance mechanism will be related to purins, efflux pumps and mutation at topoizomerazes and the deficiency of increasing efflux pumps (MFS super family and RND family) and appearance of 5 separated coding resistant genes are the resistance to disinfectants with the names of quc (A-E). on the other hand limited researches about resistant genes mechanism and secretion of different betalactamese genes that confirm strains resistance against disinfectants and drugs in hospitalsthat are related to each other directly and according to different genetically patterns they can play a crucial role in control planning and preventing the extension of the resistant isolates in hospitals infections.

Keywords: Acintobacter baumannii, beta-lactamase genes, disinfectants, medical resistance, frequency distribution of medical resistance

P161: **Efficacy of psyllium mucilage extract on pathogenic bacteria of the skin or abscesses**

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Background and Aim: Diseases, boils and pustules are common throughout humans. Particular drug is not recommended for the treatment of this disease, the most common drug in the market today is approximately a chemical drug or ointment called Black Ichthyol.

Methods: In this experiment, the methanol extract of psyllium mucilage (plantagopsylliom) was used to treat pustules and boils. Whose experiments in this laboratory on bacteria, including Staphylococcus aureus boils index ATCC/25122, Staphylococcus epidermidis ATCC /25138, Pseudomonas aeruginosa ATCC/27853, Streptococcus pyogenes was ATCC/ 8668. Disk diffusion test (Disk diffusion) or disk, or the well marked and MIC and MBC mean or average minimum inhibitory concentration MBC was used.

Results: Glaze test glazing psyllium seed only Staphylococcus aureus bacteria was influenced a concentration of 13/2 mg/ ml of 32 mm was the most sensitive zone. The extract tested for bacterial inhibition zone diameter Staphylococcus aureus and Staphylococcus epidermidis at a concentration of 40 mg/ ml, respectively, 10/5 + _0/5 mm and 13+_1mm .concentration of 80 mg /ml, respectively19/5+_0/5 mm and 18 mm.

Conclusion: The mean minimum inhibitory concentration (MIC) and the mean Minimum Lethal Concentration (MBC) respectively Staphylococcus aureus bacteria Staphylococcus epidermidis equal to 40 and 80 mg/ ml was measured. Thus, it can be concluded that the methanol extract of psyllium mucilage on bacteria, including Staphylococcus aureus and Staphylococcus epidermidis abscesses index inhibited and they can help improve this condition.

Keywords: Plantagopsylliom, Boil, Extract glaze,Bacteria,Antibacterial

P162: **Prevalence of drug Resistant Mycobacterium tuberculosis and Development of a microdilution method to evaluate drug susceptibility**

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Background and Aim: Introduction: Mycobacterium tuberculosis has developed resistance to anti-tuberculosis first-line drugs. Multidrug-resistant strains complicate the control of tuberculosis. Globally, tuberculosis (TB) still remains a major public health problem

Methods: Material and Methods: The proportion method was carried out according to the National Committee for Clinical Laboratory Standards (NCCLS) on Löwenstein-Jensen (LJ) medium. The BMM was carried out using 7H9 broth with 96 well-plates.

Results: Results: resistance was detected to isoniazid 18 (16/07%), rifampin 16 (14/28%), streptomycin 25 (22/32%), ethambutol 15 (13/39%), pyrazinamide 27 (24/10%), para aminosalicylic acid 19 (16/96%), cyclocerin 4 (3/57%) and ethionamid 14 (12/5%) cases. A total of 16 isolates were multidrug-resistant (MDR) resistant.

Conclusion: In order to prevent the spread of drug-resistant M. tuberculosis strategies for the treatment and prevention of multidrug-resistant tuberculosis are urgently required and use of rapid molecular methods are necessary.

Keywords: Keywords: Mycobacterium tuberculosis , MDR

P163: **Evaluation of antimicrobial effect of novel Quaternary Ammonium Compounds on bacteria and fungi**

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Background and Aim: Background: Quarterly Ammonium Compounds (Quat) are the more effective antimicrobial agents in medicine and industry. It needs to produce the new compounds with the wider spectrum and less toxicity, because of microbial resistance. Aim of the work was microbiological Evaluation of the new Quarterly Ammonium Compounds produced by Structural modifications on some bacteria and fungi.

Methods: Materials and methods: 16 Quat salts were designed and made in Ethanol or Aceto Nitril. Minimum Inhibitory Concentration (MIC) was determined by standard method on Nutrient Agar and Minimal agar culture media for bacteria , Potato Dextrose Agar (PDA) for fungi and Nutrient Agar and Saboro Dextrose Agar (SDA) for yeasts

Results: Results: Compounds 2,7,8,9,12,13 has the more antimicrobial effect (minimum of MIC). Furthermore, it was shown that MIC was unrelated to culture compounds. In yeast culture it must to increases the concentration in enriched media. Compounds 9,12 and 13 has the more antibacterial effect as well as antifungal effect

Conclusion: Conclusion: In comparison of structure of produced compounds and results of the work, it was revealed that radical R3 has the most important role in antimicrobial properties of Quats and it could to be substitute any suitable group related to increasing anti microbial effects.

Keywords: Quarterly Ammonium Compounds, antimicrobial effects, bacteria, fungi, MIC

P164: **The assessment of PCR test in distinction of instances brucellosis in human and it's compare with standard serological methods**

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Background and Aim: Brusellosis is one of the most dangerous infectious zoonotic diseases which at present affects most areas of Iran directly or indirectly. This disease is caused by bacteria of the genus Brusella which has7 specieses(B. abortus ,B. melitensis , B.maris B. suis ,B. canis B. ovsi and B neotomae). 4 specieses(B. abortus ,B. melitensis ,B. suis ,B. canis). Can result in clinical and subclinical disease in human which is characterized by fever, trembling, loss of weight, anorexia, joint or muscular pains, sweating, arthritis and sometimes lymphadenopathy, hepatomegaly, osteomyelitis and endocarditis. The aim of present study was determination of the rate of morbidity to human brucellosis in west Azarbaijan province

Methods: At first, human serum samples suspect of brusellusis were collected from clinics and subjected to Rose bangal and Agglutination wright,s (SAT) tests in tube. Then these samples subjected to PCR ie at first, DNA was extracted by cinagen kit and DNA precipitation subjected to PCR test by the kit of Fermentas(PCR, Cor kit) and by primer I and II pertaining to B4 and B5 gene specific for Brusella abortus. PCR products were subjected to electrophoresis on 3% gel agarose and resulted bands were compared with positive control sample (DNA obtained from Brusellea abortus culture) and marker(SM1211#).

Results: The result of this study showed that from of 53 samples of human samples suspected of brusellosis, 42 samples(79%) were positive with titer 1: 80 and higher (1: 2560) and the rest had titer less than 1: 80. In order to diagnosis confirmation, all of samples were subjected to PCR that 28samples (titer 1: 80 and higher) and 3 samples(titer less than 1: 80) were agreement with wright,s test and 14samples(titer 1: 80 and higher), 8samples(titer less than 1: 80) were disagreement with wright,s test

Conclusion: The results of this study was rerealed that with regard to brucellosis, con siderable percent of submitted samples were positive and positivity of them were confirmed with PCR test so that 58% agreement was observed amangst SAT and PCR results

Keywords: Brusellosis, Wright,s test, PCR, West Azerbayjan

P165: **Evaluation of Minimum Inhibitory Concentration (MIC) plant Essence Zataria multiflora against Acinetobacter baumannii**

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Background and Aim: Acinetobacter baumannii is one of the pathogenic bacteria in the nosocomial infection. Increased use of antibiotics against infections caused by these bacteria is the cause of drug resistance in bacteria. So the more extensive studies done on the effectiveness of new antimicrobial drugs. The aim of this study was to Evaluation of minimal inhibitory concentration (MIC) plant essence Zataria multiflora against Acinetobacter baumannii.

Methods: Zataria multiflora ingredients by GC mass measured and with 100% concentration was used. To determine the minimum inhibitory concentration (MIC) Zataria multiflora plant essence and ciprofloxacin, we use broth macrodilution method recommended by the NCCLS. In this study, the standard strain of Acinetobacter spp baumannii (ATCC 19606) was used.

Results: The results of this experiment showed that the MIC values obtained for essence Zataria multiflora 7?g and MIC values obtained for ciprofloxacin was 16?g.

Conclusion: Due to increasing drug resistance to conventional antibiotics in Acinetobacter baumannii using medicinal plants can be a good alternative for treatment.Essential oil Zataria multiflora has antimicrobial effects against strains of Acinetobacter baumannii but in practice it is necessary to study more.

Keywords: Zataria multiflora – ciprofloxacin-MIC - Acinetobacter baumannii

P166: **Evaluation of screening for methicillin-resistant Staphylococcus aureus (MRSA) using molecular loop-mediated isothermal DNA amplification (LAMP) and various PCR methods**

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Background and Aim: Staphylococcus aureus is an important human pathogen. The bacteria responsible for many kinds of infections, including surgical site infections, bloodstream infections, soft tissue infections and ... Which is the high rate of mortality. This is due to mutations in some genes of bacterial strains resistant to methicillin (MRSA) has been created. Rapid diagnosis of infections caused by MRSA in appropriate antibiotic treatment is necessary.therefore, The aim of this study was to evaluate of screening for MRSA using molecular LAMP and PCR methods for rapid detection and timely treatment infection to prevent and subsequent outcomes and mortality from it.

Methods:: This study utilizes an review of the Digital Library of Medicine database inlm, Web of Science, JAMA, SID, Springer, PubMed, Google scholar, Science Direct, and from 2009 to 2013 have been written. Accordingly, all articles with an emphasis on keywords PCR, LAMP, MRSA and MRSA detection by LAMP and PCR is selected. A total of 25 studies were selected based on a summary of the 15 studies were excluded as inconsistent with the target. 2 The full text article is not available because they only briefly in the present study were used. At the end of all articles on a variety of PCR and LAMP methods were compared Participated in the study.

Results:: Among the various articles, 4 were selected randomly. Accordingly, the sensitivity of LAMP compared Duplex real-time polymerase chain reaction (DRT-PCR) in bloodstream infections at 63 ?C for spa, mec A genes 92.3% and 96.2% ,100% and 100% specificity 100% and 100% positive predictive value (PPV) and 96.9% and 98.4% negative predictive value (NPV) . in bloodstream compared PCR to 58.5 ?C temperature for spa and arc C genes , 100% and 100% sensitivity, 100% and 100% specificity, 100% and 100% PPV and 100% and 100% NPV. The MRSA isolates from food at 65 ?C for 16srRNA, mecA, and fem A genes the 100%, 92.3% and 98.1% PPV, and 100%, 94.3% and 98.5% NPV. In certain strains of MRSA at 63 ?C for fem B, mac A genes ,100% and 100% specificity are observed.

Conclusion:: Due to the closeness of the results of LAMP and PCR, mean temperature of 63˚C for tests of short duration testing LAMP (up to 2 hours), a simple test, cost more than the PCR as a sensitive and reliability; LAMP method for detection of MRSA in comparison with other methods of speed and greater accuracy and This method is a promising alternative to other diagnostic methods.

Keywords: MRSA, LAMP, PCR,Diagnosis MRSA by LAMP,Diagnosis MRSA by PCR

P167: **Prevalence of the genes encoding for Extended-Spectrum Beta Lactamases and Aminoglycoside Modifying Enzymes and Integrons in resistant clinical isolates of Salmonella spp.**

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Background and Aim: Salmonella is recognized as an important food-borne pathogen in humans. Strains of Salmonella sp. that producing Extended-Spectrum ß-Lactamases have become a concern in medicine as regards both antimicrobial treatment and infection control in worldwide. The resistance of salmonella sp. to aminoglycosides is can due to enzymatic detoxification of them. Integrons have a important role in mobilization of resistance genes among gram negative bacteria .The objectives of this study were to determine the susceptibility of clinical isolates of Salmonella sp. and to screen the resistant isolates and detect the presence of the blaCTX-M-1، blaTEM and blaSHV genes and so the aac(6′)-Ib, aac (3)-IIa , aph(3′) -Ia , ant (2″)-Ia genes. Also we investigated carriage of class 1,2 and 3 integrons among all our isolates.

Methods: In this study, 110 Salmonella isolates collected from four hospitals in Tehran between 2008 to 2013. Susceptibility to antibiotics was determined using Kirby-Bauer disc diffusion method as per the guidelines of Clinical and Laboratory Standard Institute (CLSI). ESBL production was confirmed by combined disc test. Presence of the recommended genes was determined using specific primers and PCR.

Results: A total of 110 isolates were collected from four hospitals in Tehran. It was observed that 4.5 % of the isolates were from the blood while 95.5 % were from the stool. In this study, 62 (56.4 %) of 110 Salmonella isolates identified as serogroup D,as well as 16 (14.5 %), 11(10 %) , 2(1.8 %) and 20 (18.2 %) of the isolates belong to B , A , C, Sal spp. serogroups , respectively, based on serotyping by slide agglutination method.The rate of drug resistance of strains to 16 antibiotics was obtained. 4 isolates(3.6%) were identified ESBL producing Salmonella spp. by Combined disk method. The frequency of TEM, SHV, CTX-M-1, CTX-M-9 and CTX-M-2 enzymes were 3.6 %, 0 %, 3.6%, 0 %, 0 %, respectively. 3 isolates were resistant to the tested aminoglycoside antibiotics and the frequency of ANT(2?)-Ia , APH(3?)-Ia , AAC(6 ?)-Ib and AAC (3)-IIa were 0.91% , 0% , 50% and 0% ,respectively. The frequency of integrons class 1, 2 and 3 were 32.7 %, 6/4 % and 0 %, respectively.

Conclusion: This study showed low resistance rates to most of the clinically available antimicrobial agents for the treatment of infections caused by Salmonella spp. The resistance rates to some antibiotics, especially the drugs choice for therapy of invasive Salmonella infections in human, were increasing. In accord with other studies third-generation cephalosporins, fluoroquinolones and imipenem are suggested to be used as frontline remedial antibiotics in treatment of Salmonella infections. There is an important relationship between the presence of class 1 integron and multidrug resistance in clinical isolates of Salmonella spp.

Keywords: Salmonella, ESBLs , Aminoglycoside resistance , TEM, SHV, CTX-M

P168: **Prevalence of the genes encoding for Extended-Spectrum Beta Lactamases and Aminoglycoside Modifying Enzymes and Integrons in resistant clinical isolates of Enterobacter spp.**

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Background and Aim: The genus Enterobacter is more specifically a nosocomial opportunistic pathogen and is sought out to be one of the many key causes for extraintestinal infections next to E. coli. Strains of Enterobacter spp. that producing Extended-Spectrum ß-Lactamases have become a concern in medicine as regards both antimicrobial treatment and infection control in worldwide. The resistance of Enterobacter spp. to aminoglycosides is can due to enzymatic detoxification of them. Integrons have a important role in mobilization of resistance genes among gram negative bacteria .The objectives of this study were to determine the susceptibility of clinical isolates of Enterobacter sp. and to screen the resistant isolates and detect the presence of the blaCTX-M-1، blaTEM and blaSHV genes and so the aac(6′)-Ib, aac (3)-IIa , aph(3′) -Ia , ant (2′) -Ia genes. Also we investigated carriage of class 1, 2 and 3 integrons among all our isolates.

Methods: In this study, 110 Enterobacter isolates collected from four hospitals in Tehran between 2011 to 2013. Isolates were identified by conventional methods and by the API 20E system. Susceptibility to antibiotics was determined using Kirby-Bauer disc diffusion method as per the guidelines of Clinical and Laboratory Standard Institute (CLSI). ESBL production was confirmed by combined disc test. Presence of the recommended genes was determined using specific primers and PCR.

Results: A total of 110 isolates were collected from four hospitals in Tehran. It was observed that 78.2% of isolates belong to E.cloacae and 13.6% and 8.2% of isolates belong to E.aerogenes, E.sakazakii, respectively.It was observed that 74.55 % of the isolates were from the urin culture, 13.64% were from the chip throat, 6.36 % were from wound, 3.64% were from the blood culture and 0.91 % was from the eye infection.The rate of drug resistance of strains to 14 antibiotics was obtained. 4 isolates (12.7%) were identified ESBL producing Enterobacter spp. by Combined disk method. The frequency of TEM, SHV, CTX-M-1, CTX-M-9 and CTX-M-2 enzymes were 85/7 %, 7.1 %, 64.3 %, 0 %, 0 %, respectively. 25 isolates were resistant to the tested aminoglycoside antibiotics and the frequency of ANT (2?)-Ia, APH (3?)-Ia, AAC (6?)-Ib and AAC (3)-IIa were 20.8%, 20.8%, 54.2 % and 33.3 %, respectively. The frequency of integrons class 1, 2 and 3 were 40%, 7.3 % and 0 %, respectively.

Conclusion: This study showed High resistance rates to most of the clinically available antimicrobial agents for the treatment of infections caused by Enterobacter spp. The resistance rates to some antibiotics, especially the drugs choice for therapy of invasive Enterobacter infections in human, were increasing. In accord with other studies third-generation cephalosporins, fluoroquinolones and imipenem are suggested to be used as frontline remedial antibiotics in treatment of Enterobacter infections. There is an important relationship between the presence of class 1 integron and multidrug resistance in clinical isolates of Enterobacter spp.

Keywords: Enterobacter, ESBLs, Aminoglycoside resistance, Integrons, SHV, CTX-M

P169: **Prevalence of Klebsiella pneumoniae strains and drug resistance of these strains in patients with urinary tract infection in the city of Marand – Iran**

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Background and Aim: Urinary tract infection (UTI) is one of the most common infections in all age groups that the lack of timely diagnosis and treatment can be associated with severe complications which can ultimately lead to more advanced disease . Urinary tract infections in the United States annually, about 4/2 to 8/2 percent of children are caught and causing more than 1/1 million medical visits in years. Resistant strains of Klebsiella pneumoniae have a high prevalence, especially resistant to several antibiotics from different drug classes.This resistance is so often exacerbate the disease. The purpose of this study was assessment the pattern of resistance in Klebsiella pneumoniae isolated from antibiotic urinary tract infections (MDR) and determines the prevalence of drug resistance.

Methods: A total of 1232 urine samples were studied during a year and 60 Klebsiella pneumoniae isolates and their antibiotic resistance was determined by disk diffusion method.

Results: Klebsiella pneumoniae was isolated in 60 cases. Of these 40 isolates belonged to men and 20isolates belonged to women .

Conclusion: Alarming prevalence of urinary Klebsiella pneumoniae isolate is notice for more attention to the proper administration of antibiotics in cases of UTI.

Keywords: Klebsiella pneumoniae,Urinary Tract Infections, drug resistance

P170: **Evaluation of Induced IgM, IgA Titer of D-LPS-Exotoxin A Conjugated against P.aeruginosa**

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Background and Aim: P.aeruginosa is one of the most common nosocomial infections especially in immunocompromised patients. This pathogen causes life-threatening symptoms in humans. Although antibodies directed to bacterial Lipo- polysaccharides (LPS) are protective ,these antigens are variably immunogenic. The immunogenicity of LPS antigens has been enhanced by covalent coupling to proteins to form LPS-Proteins conjugate vaccines.So in this study we evaluated induced and Titers of D-LPS-Exotoxin A conjugated against P.aeruginosa.

Methods: LPS of P.aeruginosa was isolated, purified and detoxified by NaOH . For conjugation of to Recombinant Exotoxin A ,was linked ADH as a spacer molecule and EDAC as a linker and then passed through a column of CL-2B sepharose in saline.The prepared antigens were injected to animal model intra peritoneally.Vaccination was performed by 3 doses. Then serum samples were collected and antibodies response against LPS was measured by Antigen Mediated Elisa method for IgA and IgM.

Results: Antibodies titers were shown that third inoculation of antigen for D-LPS- Exotoxin A and D-LPS has enhances antibody titers for . Also Exotoxin A was shown the highest titers in all antibodies .

Conclusion: D-LPS- Exotoxin A increased immunogenicity in animal model as compared with the unconjugated D-LPS. So conjugates of P .aeruginosa will be useful method for vigorous infections of pseudomonas.

Keywords: P.aeruginosa, conjugate,D-LPS, D-LPS- Exotoxin A , Antibody,Titer

P171: **The effect of CHLOROFORM EXTRACTS OF GERMAN CHAMOMILE on Escherichia coli infected mice**

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Background and Aim: Some strains of E.coli are considered the main pathogens of humans and animals. Emergence of new strains of this organism with the ability of resistance to a wide range of antibiotics, especially beta-lactams necessitates the efforts to search alternatives for better treatments to deal with these infections. Chamomile is one of the most widely used herbs in the world. In-vitro antimicrobial properties of Chamomile plant on a range of different organisms are reported in the literature. In this study we investigated the potential therapeutic effects of chamomile chloroform extract on E. coli intra-peritoneal infection of BALB/c mice.

Methods: 64 female BALB/c with an average weight of 20 to 25 g, were divided into 8 groups. Lethal dose of the E.coli for mice (109 CFU/mL) was injected intraperitoneally in test groups and treated with 5.25, 10.5 and 21 mg/mL of chamomile chloroform extract. The combined effect of the extract, the highest dose of the effective extract with amikacin (50 µg/kg) and amikacin alone were also examined.

Results: The highest number of survived mice at the dose of 10.5 mg/mL of extract and the highest death rate with 5.25 mg/mL of extract was observed. In amikacin group all infected animals were survived. Mortality rate in chamomile extract combination with amikacin is almost the same as amikacin alone. The lowest mortality rate was observed in the group which had been treated with a combination of extract and amikacin.

Conclusion: The result of this study does not recommend in-vivo using of chloroform extracts of chamomile for E.coli infection via intraperitoneal administration. In vivo chloroform extracts of chamomile, itself seems not to have a strong antibacterial effect on on E. coli intra-peritoneal infection of BALB/c mice.

Keywords: chamomile extract , Escherichia coli , Amikacin , BALB/c mice

P172: **Serologic Examination of Hepatitis B in Eastern Azerbaijan during 1391**

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Background and Aim: Hepatitis B is considered as one of the most common infectious diseases. Contamination with this virus has caused hygienic problems all over the world. Liver cirrhosis and hepatocellular carcinoma are the most important consequences of this disease. Regarding to the ways of hepatitis B transmission and its prevention, detecting positive cases and carriers, hepatitis B is of especial importance. The objective of this study is to determine the rate of hepatitis B infection in Eastern Azerbaijan province.

Methods: This study was carried out on partial and short-time basis, during Iranian calendar year 1391, using results of 24180 patients, whom had been visited in clinics of Eastern Azerbaijan. The most regular laboratorial detection methods were serologic methods such as ELISA, chemiluminescence, electrochemiluminescence. HBsAg of the samples was analyzed by the aid of chemiluminescence method.

Results: Among 24180 patients, 462 samples (1.9%) were positive and 23718 samples (98.1%) were reported negative; total 462 positive cases included 268 (58%) female and 194 (42%) male patients.

Conclusion: According to the results, gender is not a determining factor affecting the susceptibility of patients. This virus is epidemic, so diagnostic and preventive methods are crucial. Moreover, comprehensive national vaccination can help to noticeably reduce the infections caused by this disease.

Keywords: HBsAg, Hepatitis B, Eastern Azerbaijan, chemiluminescence

P173: **Survey of effect of Magnetospirillum gryphiswaldense in animal like-thalassemia: serum iron, total liver iron & iron urine**

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Background and Aim: The magnetotactic bacteria Magnetospirillum gryphiswaldense (MSR-1) mineralizes the magnetite (Fe3 O4) crystals and organizes a highly ordered intracellular structure, called the magnetosome. Iron transport system supports the biogenesis of magnetite. Iron is an essential element for most life on Earth, including humans, but if the level of iron in body increases, it can cause many disorders e.g. Thalassemia. The aim of this study was investigation on effect of bacterial magnetite formation that takes up iron from the blood in animal model of thalassemia.

Methods: In this study, Balb/c mice were treated with iron dextran had high hepatic iron loading to achieve the iron–storage level. After test treated of iron, MSR-1 were injected to iron overloaded mice. Viable, bacterial number was determined in special organs such as liver, spleen and lymph nodes by measuring colony-forming units (CFUs). Moreover, serum iron levels were tested by using commercial kites, total iron in liver were measured after wet ashing and analyzed for total iron excreted using flame atomic absorption spectrophotometer.

Results: After i.p administration of iron dextran in mice, serum iron and total liver iron were increased. According to CFU measurements, after 96 hours mice can clear MSR-1 from its body. We have also shown that MSR-1 bacteria can effect on the blood iron level in iron- overloaded mice. The serum iron levels have been decreased compare with control level to 10 days ($P< 0.05$), the total liver iron levels in liver have been significant decreased compare with control level during the first 3 days ($P< 0.05$) and total iron excretion have been significant increased compare with control level to 8 days after MSR-1 i.v injection.

Conclusion: This study provides the base on another study that it is offered new application for MSR-1 in iron-overloaded disease. MSR-1 cells have the ability to decrease iron values in iron-overloaded mice, and therefore inhibit the possible damage to different body organs caused by iron overloading.

Keywords: Magnetospirillum gryphiswaldense; Changes Iron Level; Animal like-thalassemia

P174: **Proteomic Comparison of Cyclamen coum extract Treated- P. aeruginosa Biofilm with untreated Biofilm**

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Background and Aim: Biofilm formation is a major pathogenesis determinant in different bacteria such as Pseudomonas aeruginosa. Some studies revealed that biofilm dramatically increases bacterial resistance to several antibiotics. In order to re-sensitize bacterial biofilms to antibiotics, biofilms should be dispersed. In this study, the effect of n-butanolic Cyclamen coum extract on pre-established biofilm of P. aeruginosa was examined.

Methods: The biofilm formation by P. aeruginosa PAO1, P. aeruginosa 8821M and 4 clinically isolated P. aeruginosa was confirmed by microtiter plate method and PCR molecular technique (amplifying cup A gene which is involved in biofilm formation). Extraction of the tubers of Cyclamen coum was done by fractionation method by applying different solvents including petroleum ether, ethanol and n-butanol. The effect of n-butanolic C. coum extract (which includes saponin compounds) on planktonic cells, 1-day-old and 3-day-old P. aeruginosa biofilms were examined by agar well diffusion and crystal violet methods. Whole protein expression profile of treated and untreated P. aeruginosa PAO1 biofilms were separated by two-dimensional (2D) gel electrophoresis and the differentially expressed proteins were identified by MALDI-TOF mass spectrometry.

Results: The biofilm formation by P. aeruginosa strains was quantitatively confirmed. The PCR method confirmed the existence of cup A gene (172 bp) in all studied strains. Saponin content of the n-butanolic C.coum extract was 156 µg/ml. The extract revealed antibacterial activity against planktonic P. aeruginosa strains. The n-butanolic C. coum extract significantly affected 1-day-old and 3-day- old biofilm mass ($p<0.05$). By comparing the 2D proteomes, a total of 400 protein spots in Cyclamen coum treated- P. aeruginosa biofilm were detected. According to two-dimensional (2D) gel electrophoresis results, the proteins involved in metabolism and bacterial antibiotic resistance were down regulated in treated P. aeruginosa biofilms.

Conclusion: This proteomic analysis provides a fundamental platform for further studies to reveal the role of saponin as P.aeruginosa biofilm dispersant.

Keywords: Pseudomonas aeruginosa, Biofilm, Cyclamen coum, two-dimensional (2D) gel electrophoresis

P175: **A Fast and Accurate method for Cholera Diagnosis Using Fecal Specimens on DBC**

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Background and Aim: Vibrio cholera is one of the important worldwide gastrointestinal water-borne significant diseases. Cholera outbreak have been reported from different parts of South America, Asia (Especially in India and Bangladesh), and south Africa affected millions of people .In according to reported the World Health Organization (WHO) in 2010 the cumulative number of cases represented an increase of 43% compared to the number in 2009, and an increase of 130% compared to that in 2000. Accordingly, rapid and accurate diagnosis of cholera prevents the disease from being an epidemic. This abstract describes a new method for laboratory rapid diagnosis of cholera by means of Feces sample spotted on filter paper and PCR assay.

Methods: In this study, we used of artificially stool sample infected by Cholera to examine the accuracy of the new method. Two sets of samples were prepared, the first samples of bacterial suspensions (107 - 108 cfu ml−1) with 3-fold serial dilutions and the second samples of 3-fold serial dilutions mixed with equal amounts of stool samples. Both sets of sample spotted on separate DBCs and dried at room temperature. The two discs (1 mm diameter) were prepared from each card after washing and both were investigated, eluted DNA by molecular technique of PCR. We used a pair of specific primers from 16S-23S rRNA intergenic spacer regions among Vibrio species.

Results: Tests result showed that the bacterial suspension sample dilution (10-2) and the artificially cholera stool sample dilution (10-2) both had sufficient amount of DNA for PCR Assay. These results of PCR amplification on DBC card showed a suitable medium for transportation cholera Feces samples.

Conclusion: This method has considerable advantages in compare with routine method including safe and easy samples transportation, reduced environmental contamination, Fast DNA Extraction without PCR inhibition and the most important, prompt diagnosis

Keywords: DNA Banking Card (DBC), Bacteria Suspension, 16S-23S rRNA intergenic spacer regions

P176: **Prevalence of antimicrobial resistance in Helicobacter pylori isolates from Iranian samples and other countries**

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Background and Aim: Helicobacter pylori, a class I carcinogen, is a main causative agent of chronic gastritis, peptic ulceration and gastric carcinoma. The prevalence of antibiotic resistant H. pylori strains may be variable. So, it seems necessary to investigate the drug resistance of H. pylori in different areas to choose the best treatment options in patients. The aim of this study is to assess the prevalence of drug resistance in H. pylori clinical isolates from patients in Iran and others countries. In fact this study we compare antibiotic resistant H. pylori in Iran and other countries.

Methods: In this study we have attempted to review papers on antimicrobial resistance in H. pylori and comparison bacterial resistance in Iran and other countries.

Results: This study shows a high level of the prevalence of metronidazole resistance in Iran,while in Japen among 12 clarithromycin-resistant strains studied, 11 strains (92%) showed cross-resistance to azithromycin. Considering previous studies in Iran , rate of resistance reported 35 and 54.16% for metronidazole, and also 2.4 and 4.16% forclarithromycin, and 2.45 and 8.3% for amoxicillin studies, no resistance to tetracycline and furazolidone was reported .Therefore, the major antibiotic resistance would be associated with metronidazole in this area. In Brazil, resistance to amoxicillin has been detected in 29% of strains recovered posttreatment. Recent studies have reported that clinical isolates from children are clarithromycin resistant, with rates of resistance ranging from 18 to 44.8%. However the prevalence rate of increase in resistance, particularly from Europe to Asia, America, Africa. Metronidazole resistance rate in Asia, Korea was the highest and in Japan was lowest.

Conclusion: As the antibiotic susceptibility testing is not routine for H. pylori isolates in Iran, the empirical treatments are usually used in clinical therapies and there is a risk of increase in drug resistance in the future. It is important for specialized centers to perform the monitoring of antibiotic resistance to define appropriate and specific treatment patterns in our country

Keywords: Antimicrobial resistance, Helicobacter pylori, Iran

P177: **Comparison of PCR and Loop mediated isothermal amplification (LAMP) techniques in detection of Pseudomonas aeruginosa Keratitis**

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Background and Aim: Pseudomonas aeruginosa is an opportunistic bacterium and can involve almost any organ or part of the body. Pseudomonas can cause devastating infections in humans and is one of the most common causes of bacterial keratitis. For detection of Pseudomonas keratitis can be used to classic methods such as culture, but this method has low sensitivity in diagnosis. Given the importance of the patient's disease and prevent blindness, use of molecular methods have been discussed such as PCR for rapid detection with high sensitivity. The aim of this study is to compare Golden PCR technique with Loop mediated isothermal amplification (LAMP) techniques in detection of Pseudomonas aeruginosa Keratitis.

Methods: After designing the primers used in PCR and LAMP, optimized both tests and sensitivity and specificity were determined. In this study, 45 samples of suspected Pseudomonas keratitis of corneal Labbafinejad hospital patients were collected by the physicians. All DNA samples were extracted by the method of boiling and DNG-PLUS and the samples were tested by PCR and LAMP. Finally, PCR results were checked by electrophoresis and LAMP test results were evaluated by SYBR-green and electrophoresis.

Results: The results showed that both LAMP and PCR methods are able to specifically diagnose of Pseudomonas aeruginosa and in specificity test, with none of the samples was amplified products. Sensitivity of PCR and LAMP test were achieved 50 and 1 CFU respectively. Appropriate extraction method to extract the DNA of the pseudomonas from keratitis is DNG-Plus method. The number of positive samples in the LAMP and PCR methods were, respectively, 17 and 6 samples.

Conclusion: According to the comparison PCR with LAMP results can be expressed that the LAMP test, despite the simplicity and lack of need for advanced equipment have been more sensitive than PCR reaction and can be considered a suitable alternative to the PCR. Using this technique will provide the possibility molecular diagnosis with high sensitivity for early detection of infection with Pseudomonas keratitis in all diagnostic centers without the need for advanced equipment with minimum cost.

Keywords: keratitis, PCR, LAMP, Pseudomonas aeruginosa, Comparison

P178: **Comparison of LAMP and PCR techniques for detection of Legionella pneumophila in hospital and environmental water**

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Background and Aim: Legionella pneumophila is a causative agent of Legionnaires' disease. Among the Legionella species, L. pneumophila is associated with 90% of human disease. Rapid and precise detection of Legionella in water systems is of the most importance for risk prediction and the elimination of Legionella from possible infection sources. The best detection system for this purpose is molecular methods. Among molecular methods we choose PCR and LAMP and then comparison the result of these tests. The aim of this study is attaining the most sensitive, fastest and cheapest method for detection and identification of L. pneumophila in environmental and hospital water sample.

Methods: In this study we design specific PCR on the basis mip gene of L. Pneumophila and then according to the product of the PCR test design 6 specific LAMP primer with primer explorer V.4 software. After of PCR and LAMP optimization, carry out sensitivity and specificity tests. Amplicon was cloned and sequenced by Dideoxy chain termination. 115 water samples were collected from environment and hospitals. Samples divided in 5 groups: 1) tap water, 2) hospital water, 3) water cooler 4) Sewage 5) Stagnant water. Samples DNA extracted by simple boiling method.

Results: The product of optimized PCR with 627 bp length correctly amplified and observed on electrophoreses gel, also LAMP test was optimized with the Bst Large fragment DNA polymerase in 66 degree temperature and 60 min. Evaluation of the selected primers with 10 various DNA demonstrated 100% specificity for both PCR and LAMP test . Sensitivity of the test was 1 CFU for LAMP test and 10 CFU for PCR. Results from 5 groups were noticeable. A total LAMP test was positive of 44 samples from 115 subjects in five groups (38.26%) and for PCR test was positive of 9 samples (7.8%).

Conclusion: The results showed that the LAMP technique is more sensitive than PCR for diagnosis of Legionella pneumophila .

Keywords: Legionella pneumophila, Loop mediated isothermal amplification, PCR, Diagnosis

P179: **Evaluation of the PCR and LAMP Techniques in the Diagnosis of Adenovirus Keratitis**

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Background and Aim: Adenovirus is one of the most important factors of keratoconjuncitivitis , gastroenteritis, respiratory tract infections and cystitis. Serologic methods can be used to detect this agent but there are limitations with them. At the same time, Molecular methods are much reliable for adenovirus detection. So, in this study PCR and loop mediated isothermal amplification (LAMP) was used in order to diagnose Adenovirus in suspected samples of Adenoviral keratitis patients.

Methods: In this study, 86 suspected patients to Keratitis have been examined to compare the PCR with LAMP method. By use of specific needles, biopsies were collected from surface of the cornea. DNA samples of patients have been extracted using DNG-Plus. After of tests optimization, sensitivity and specificity of two tests have been done for LAMP and PCR, and then used for the samples.

Results: PCR product with 301 bp length was detected and confirmed on agarose gel electrophoresis and by sequencing. PCR sensitivity for adenovirus was 10 particles and specificity was 100%. From the 86 samples, 27 samples (31%) showed positive result. LAMP sensitivity was determined as 4 particles in this study compared with 10 particles by PCR. In total, 28(33%) out of 86 samples were identified as positive by LAMP, whereas 27(31%) out of 86 were identified as positive by PCR test.

Conclusion: Using LAMP technique to diagnose adenovirus showed that isothermal proliferation technique by LAMP has more sensitivity and precision. In addition, this technique provide a faster and more trusting method for diagnosing infections such as Keratitis that there is an emergency need to be diagnosed to be cured.

Keywords: Adenovirus, LAMP, Keratitis, PCR

P180: **Samples sent to the Central Laboratory of the incidence of cholera in Zahedan, the years 1386 till 31 Khordad 1392**

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Background and Aim: By a bacteria Vibrio cholera of cholera disease that is caused through the water. This disease in In the past is disrespecting named read Mrgamrgy. These bacteria with drinking contaminated water or eating uncooked fish or oysters enters the body by eating. of cholera transmission in Iran most ways, Contaminated vegetables.Epidemic cholera had made a lot of that is due to always been top of it mortality causes the international concern from of public health view is. Recorded in Iran of 45 years before the the incidence of the epidemic continuous with the dispersed there are. This study examines the latest situation of cholera in samples sent to the Central Laboratory of Zahedan in the years 1386 to 31 khordad1392 deals.

Methods: In a cross-sectional study and based on the data collected from the Central Laboratory of Zahedan In a 6-year period between 1386 to 31 khordad 1392 age range and gender were divided into two groups, the incidence of cholera were studied in each group.

Results: The survey carried out in the number of 77 cases of cholera have been reported between the years 1386 till 31 Khordad 1392, Of these individuals 49 Person(63%) patients were in the age group 15 to 35 years, 42 Person (55%) patients Iranian nationals, of whom 35 (45%) were non-Iranian nationals. 66 Person(86%) males and 11 (14%) were female. from 42 Iranian nationals who were 32 Person(76%) residing in urban areas and 10 patients (24%) resident in rural areas.

Conclusion: Approximately half of patients with cholera non-Iranian nationals who have illegally crossed the border boundary camp are held Through some identification and number it were entered the and Suburbs city of Zahedan, Also were a few in the hospital in Zahedan Bu ali, According to the Iranian patients residing urban areas were the most, And according to the findings lack pollution and drinking water networks in favor the transmission of cholera are shunted through person to person, We conclude that main cause of the cholera the outbreak in Zahedan those who illegally crossing borders and enter Iran were, caused by Unorganized countryto being eastern borders, Countries such as Afghanistan and Pakistan are due to economic difficulties, health, political and recent natural disasters have the ability to eradicate communicable diseases such as cholera do not have, So should borders between the two countries, particularly in the form of control.

Keywords: cholera, Vibrio cholerae, Zahedan

P181: **Construction a latex agglutination test for differential and rapid diagnosis of vaginitis**

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Background and Aim: Vaginitis is the most common gynecologic diagnosis in the primary care setting. Three major pathogens of vaginitis are including Candida spp., some bacteria and Trichomonas vaginalis. Differntial diagnosis of vaginitis is important since its misdiagnosis usually leads to use of multi-drug treatment. Considering low sensitivity of microscopic methods and time consuming for culture methods, in this study construction of a latex agglutination test for rapid diagnosis of the disease has been investigated.

Methods: In this study Antibody against Trichomonas vaginalis and Candida albicans raised in rabbits. Salting out method was used for antibody purification. Purified antibodies were conjugated to latex particles. Then this latex particles were mixed with different dilution of Trichomonas vaginalis or Candida albicans.

Results: Following mixing latex particles conjugated with anti Trichomonas antibody with Tricomonas vaginalis, agglutination was detected. Also following mixing latex particles conjugated with anti candida antibody with Candida albicans agglutination was detected

Conclusion: considering results of this study, more investigation is recommended to use latex agglutination test in vaginitis diagnosis.

Keywords: latex agglutination,Trichomonas vaginalis,Candida albicans ,vaginitis

P182: **Effect of Taxus baccata extract and fractions on Trichomonas vaginalis growth**

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Background and Aim: Tichomoniasis is a very common sexually transmitted disease (STD) that is caused by infection with a protozoan parasite called Trichomonas vaginalis. Metronidazol with vast side effects is now the only drug approved for the treatment of this infection. In an attempt to find an alternative drug, the effect of Taxus baccata on several parasite was shown in previous studies. In this investigation, the effect of different extracts of this plant on T. vaginalis in culture medium has been investigated.

Methods: Dichloromethan extract of leaves of Taxus baccata and its 60% and 90% fractions of it were prepared. The extracts and its fractions were then dried using a rotary evaporator. The effect of the extract and fractions on Trichomonas vaginalis was g following 24,48 andμtested in concentrations of 200, 300, 400 and 500 72 hours in culture medium.

Results: Outcome data of this investigation revealed that in all concentrations crude extract and 60% fraction had stronger anti-parasitic effects in comparison to 90% fraction.

Conclusion: Considering the appropriate effect of this plant on Trichomonas vaginalis, more investigation is recommended to convert this plant to a an anti Trichomonas vaginalis drug.

Keywords: Taxus baccata,fractions, extract,Trichomonas vaginalis

P183: **Evaluation of Antibiotic Resistance Profile of Gram Positive Bacteria Isolated from Inpatient of Academic and Privet hospitals in the Arak, Iran. 2011-2012**

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Background and Aim: Our country's per capita consumption of antibiotics is a significant and sometimes illogical and the per capita consumption has increased considerably in recent years. The purpose of this study is to investigate the patterns of antibiotic resistance of gram positive bacteria in both academic and private hospitals.

Methods: This descriptive cross – sectional study since the beginning to for one year (June 2011 to June 2012), including all cases referred to AmirAlmomenin hospital (academic hospital) and Imam Khomeini hospital (privet hospital) microbiology laboratories. Gram-positive organisms according to 2010 Clinical Laboratory Standards Institute (CLSI M100-S20) pattern were tested for sensitivity to important antibiotics of clinical practice by the disk diffusion method. Finally, all data were analyzed by SPSS software version 19.

Results: from the 846 specimens of patients in both hospitals, 326 samples (38.5%) were gram-positive that of these numbers were isolated 61.3% coagulase-negative Staphylococcus, 13.5% enterococci and 5.5% Staphylococcus aureus. The prevalence of gram-positive organisms were obtained in AmirAlmomenin hospital (academic Hospital) 84.4% and in Imam Khomeini Hospital (privet hospital) 15.6%. (P=0.009) Also, special resistance components were significantly more in AmirAlmomenin hospital (academic Hospital) compared to Imam Khomeini hospital (privet hospital). (P=0.001)

Conclusion: The Prevalence of gram-positive organisms isolated had a significantly different in the two hospitals and also between the profiles of special resistances there was significant differences in the two academic and privet hospitals. Therefore, the care program for review and monitoring of the regional resistance patterns is recommended.

Keywords: Resistant pattern, Antibiotic, Hospital infection

P184: **Effect of lactobacillus casei on intestinal Escherichia coli populations in mice.**

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Background and Aim: Enterobacterial infections are one of the two leading killers of children in developing countries. Enterobacterial infections can be produced by bacteria that normally live in the human digestive tract without causing serious disease, or by bacteria that enter from the outside. The most troublesome organism in this group is E.coli. Our purpose in this study determination effect of lactobacillus casei as probiotics on E. coli as intestinal microflora

Methods: Twenty mice aged 5 weeks and weighing 20±2 grams were selected. A 1-week adaptive period on a normal diet were done. The 20 mice were randomly selected and assigned to two groups of 10 rats each. The treatment group received 0.3 mL (109 CFU/mL) daily of lactobacillus casei ATCC 334 solutions intragastrically for the 28-days study period. The control group was intragastrically injected by sterile normal saline. 1 gram of each sample (cecum tissue of each mouse) were removed and transferred to a tube with 9 mL of 0.9% NaCl solution and homogenized by vortexing for10 minutes. After serial dilutions each sample was plated in EMB agar and Incubated at 37°C for 24 hours and the grown colonies were counted.

Results: The control group showed 5.7 log CFU/g E. coli and this content was 4.0 log CFU/g for treated group.

Conclusion: Our results showed that L. casei can significantly ($p < 0.05$) decreased intestinal E. coli population and can be useful in controlling these pathogen and enterobacterial infections.

Keywords: lactobacillus casei- Escherichia coli- intestinal microflora

P185: **Phytochemical Screening and Antibacterial Potentiality of Different parts of the Prosopis farcta extracts against Methicillin-resistant Staphylococcus aureus (MRSA)**

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Background and Aim: Drug resistance of microorganisms is the major global threat. Therefore entails the need to search source of drugs with high impact on microorganisms. Plant materials able to provide protection from microbes can be effectively used as a drug. The purpose of this study was Phytochemical Screening and antibacterial potentiality of different parts of the Prosopis farcta extracts against Methicillin-resistant Staphylococcus aureus (MRSA).

Methods: Preliminary phytochemical screening of the Prosopis farcta methanolic extracts was carried out for estimation of saponins, glycosides, alkaloids, tannins and flavonoids using standard phytochemical screening methods. The MRSA cultures were obtained from the microbiological laboratory of the Hospitals in Zabol (Iran). The P. farcta plant was collected from area of Hamoon wetland in Zabol. Antimicrobial activity was evaluated based on the disc diffusion method. The Minimum Inhibitory Concentration (MIC) for bacterial growth and Minimum Bactericidal Concentration (MBC) of different parts (Root, Leaf, Pod and Seed) of the P. farcta methanolic extracts were determined by microdilution technique. Determination of Vancomycin MIC was done by agar dilution method.

Results: The preliminary phytochemical analysis of different parts of the P. farcta showed that root extracts demonstrated a high concentration of flavonoids, saponins, phenols and moderate concentrations of alkaloids, tannins, glycosides and resins. Leaf and pod extracts demonstrated a high concentration of saponins and flavonoids and moderate concentrations of alkaloids, resins, glycosides, tannins and phenols. Seed extract demonstrated a high concentration of alkaloids, tannins, glycosides and moderate concentration of flavonoids, saponins, phenols and resins. All P. farcta extracts recorded different degrees of antibacterial activity on MRSA as evidenced by the zone of inhibition. Inhibition zone for root, leaf, pod and seed extracts were 5±0.1, 6±0.4, 8±06, 12±0.1 mm, respectively. MIC for root, leaf, pod and seed extracts were 45±0.4, 35.5±0.8, 15±0.1, 5±0.4 µg/ml, respectively. MBC for root, leaf, pod and seed extracts were 100±0.4, 75±0.3, 25±0.5, 5±0.2 µg/ml, respectively. During our study we observed that all strains isolated from various clinical samples had MIC of Vancomycin between 0.4-4 ?g/ml.

Conclusion: The antibacterial effects of different part of the P. farcta plant on MRSA may be due to the presence of phytochemical components. All P. farcta extracts exhibited different degrees of antibacterial activity on MRSA; this difference is mainly due to the presence of different components. Seed extract showed more effect than other part of plants. This effect may be related to high concentration of alkaloids, tannins or glycosides. These components are known to high antibacterial activity. The mechanisms thought to be responsible for the action of these phytochemicals against MRSA may include enzyme inhibition by the oxidized compounds which act as a source of stable free radical and often lead to inactivation of the protein and loss of function. They have the ability to complex not only with extracellular and soluble proteins but also bacterial cell walls and disrupt microbial membranes. However, the search for new antibacterial agents should be continued by screening many other plant families. The antimicrobial and phytochemical studies would provide valuable information to the knowledge media worldwide.

Keywords: Phytochemical screening, antibacterial potentiality, Prosopis farcta, Vancomycin, Methicillin-resistant Staphylococcus aureus (MRSA).

P186: **A study of antibacterial potentiality of some plants extracts against multi-drug resistant human pathogens**

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Background and Aim: Recently, the effects of pathogenic microorganisms and resistance against antibiotics that have acquired the properties of the extracts and compounds of biological species are the center of attention. Antimicrobial of herbal compounds are one of the most valuable resources in medicine. As a results the spread of infectious diseases, to identify more of these extracts and compounds useful in the treatment of patients. This study has been designed to determination potential of antibacterial activity leaf and seed ethanol and aqueous extracts of six plants (Tribulus terrestris, Convolvus arvensis, Malva parviflora, Melilotus indicus, Rumex chalepensis and Anchusa arvensis) against two Gram-positive (Staphylococcus aureus NCTC7428, Bacillus subtilis MTCC 441) and four Gram-negative (Pseudomonas aeruginosa MTCC 2453, Escherichia coli MTCC 739, Enterobacter aerogenes ATCC 13048 and Klebsiella pneumoniae MGH 78578) pathogenic multi-drug resistant bacteria.

Methods: The plant material used in this study consisted leafs of Tribulus terrestris, Convolvulus arvensis, Malva parviflora, Melilotus indicus, Rumex chalepensis and Anchusa arvensis, collected from area Zabol, Iran. The two Gram-positive include Staphylococcus aureus NCTC7428, Bacillus subtilis MTCC 441 and four Gram-negative include Pseudomonas aeruginosa MTCC 2453, Escherichia coli MTCC 739, Enterobacter aerogenes ATCC 13048 and Klebsiella pneumoniae MGH 78578 were obtained from the microbiological laboratory of the amir-almomenin hospital in Zabol, Iran. Antimicrobial activity was based on the disc diffusion method. The minimal inhibitory concentration (MIC) for different plants has been determined by microdultion technique.

Results: Result showed that, all the extracts of plants (ethanolic leafs extract, aqueous leafs extract, ethanolic seeds extract, aqueous seeds extract) recorded different degrees of antibacterial activity against multi-drug resistant bacteria as evidenced by the zone of inhibition. The result showed that ethanolic leaf extract compared with aqueous leaf extracts having greater antibacterial activity. Lowest MIC of ethanolic and aqueous leaf extracts related to K.pneumoniae (Anchusa arvensis L. 116.4 mg/ml), E.aerogenes (Rumex chalepensis L. 214.1mg/ml) respectively. Lowest MIC of ethanolic and aqueous seed extracts related to E.aerogenes (Tribulus terrestris L. 209.2 mg/ml), S.aureus (Rumex chalepensis L. 274.9 mg/ml) respectively.

Conclusion: The all plant used in this study, could be used to discover bioactive natural products that will lead to the development of new pharmaceutical entity such as screening of various natural organic compounds and identification of active agents must be reasonable as a productive approach in the search of new herbal drugs. The antimicrobial activities can be improved if the active components are purified and adequate dosage determined for proper administration. This may go a long way in preventing the administration of unsuitable concentrations, a common practice between many traditional medical practitioners. We also suggested that some of the plants in this study, which possesses strong antibacterial activity, in the treatment of diseases caused by the microorganisms tested.

Keywords: Plant extracts, ATCC, MTCC, NCTC, Zone of inhibition, MIC

P187: **Serologic study of Toxoplasmosis in women with breast cancer**

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Background and Aim: Toxoplasmosis, a prevalent parasitic infection among people and a variety of mammals and birds, can be acquired or congenital, and acute or chronic. Acute Toxoplasmosis is considered more susceptible in cases with immune deficiency AIDS/HIV, or cancer, or in cases taking immune suppressive medication. This study aimed at examining Anti-Toxoplasmosis Gondii antibodies and assessing its risk of infection in women with breast cancer in the city of Ilam.

Methods: In 2011, this case-control study was conducted with 120 women attending Mustafa Khomeini Hospital. 60 women with breast cancer composed the case, and 60 healthy women were in the control group. ELISA was employed to evaluate the level of IgG and IgM and data were analysed by SPSS software (16) through independent T-test.

Results: In this study, the participating women were in their discharge phase and before their menopause age. The mean age of the case group, and the control group was 40 and 41 years , respectively. Among the participating women, 30 (50%) had a CA 19-9 tumor marker. The relationship between Toxoplasma and breast cancer was found significant ($p<0.01$). The mean concentration of IgG among the women with breast cancer was 1.87%, and it was 1.09% for the ones without breast cancer, thus showing a meaningful relationship with Toxoplasmosis ($p=0.01$). Anti-Toxoplasmosis IgM in the women with and without breast cancer was not found positive.

Conclusion: Breast cancer is known as one of the most common cancers threatening Iranian women. The patients with breast tumors are threatened by opportunist infections like Toxoplasmosis. According to our findings and the meaningful relationship between chronic Toxoplasmosis and breast cancer, it is recommended that physicians pay attention to Toxoplasmosis in periodical examinations in order to prevent acute Toxoplasmosis.

Keywords: Serology, Toxoplasmosis, breast cancer

P188: **Synthesis of Curcumin Nanogel for treatment of Pseudomons aeruginosa infection in burn model**

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Background and Aim: Pseudomonas aeruginosa is an etiological agent of serious infections in patients with burn wounds. It is an opportunistic pathogen causing severe, acute and chronic nosocomial infections in burn patients. The organism is generally resistant to many antimicrobial agents due to natural resistance or mutations. Burn hospitals often harbor drug resistant P. aeruginosa that can serve as a source of nosocomial infections. Curcumin is a plant derived compound which has potential activities beneficial for the treatment infection disease. The insolubility of curcumin in water restricts its use, which can be overcome by the synthesis of curcumin nanogel.

Methods: Single emulsion –solvent evaporation method, was used for encapsulating hydrophobic curcumin, to synthesis of poly lactide glycolic acid (PLGA)-curcumin nanogel. We have successfully synthesized water-soluble PLGA- curcumin nanogel and characterized it using SEM, TGA (Thermo-gravimetric Analysis) and cytotoxicity analysis. Cytotoxicity profile of the nanogel was studied using MTT Assay. In vitro studies on P. aeruginosa were investigated by broth microdilution method, and biofilm production. The burn model was conducted on the male 200g rat and the wound was infected by the clinically isolated P. aeruginosa. The infected burn wound was treated by the PLGA-curcumin nanogel every day for one week.

Results: The synthesized nanogel was completely soluble in water and showed fluorescent properties under UV light. The nanogel are highly biocompatible and do not possess any significant toxicity in vitro on human fibroblast cells. MIC values of imipenem, Amikacin and nanogel were 8µg/ml, 6µg/ml and 1µg/ml, respectively. Nanogel effect on the infected burn model was better than imipenem and Amikacin treated mice in significant.

Conclusion: In conclusion our results suggest that curcumin encapsulated-PLGA nanogel are able to kill P. aeruginosa and is non-toxic for sensitive cells. The encapsulation of the curcumin in PLGA does not destroy its beneficial properties and can be used as a drug to be a potential therapeutic tool for infected burn patients.

Keywords: Burn wound, Curcumin, Nanogel, Nosocomial Infection, Pseudomons aeruginosa

P189: **Nanocurcumin inhibits methicillin resistant Staphylococcus aureus growth in vitro and in vivo**

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Background and Aim: Revelation of methicillin resistant Staphylococcus aureus (MRSA) requires the exploration of new and/or improved antibacterial therapies. So we started to study the effect of Nanocurcumin on MRSA. Colonization of the MRSA in cardiac patients has become endemic in many hospitals and care centers in the Iran and developing countries. The bacteremia and endocarditis caused by MRSA are devastating infections associated with high mortality rates (25%) of affected patients. In this study, curcumin that is used in traditional Chinese medicine has been evaluated for its anti-MRSA activity in vitro and in a rat model as encapsulated form in poly-lactide-glycolic acid nanoparticles (Nanocurcumin) to increase its solubility and availability.

Methods: Single emulsion–solvent evaporation method, was used for encapsulating hydrophobic curcumin, to synthesis of poly lactide glycolic acid (PLGA)-curcumin nanoparticles. We synthesized water soluble Nanocurcumin and characterized it using scanning electron microscopy and cytotoxicity analysis. Cytotoxicity profile of the Nanocurcumin was studied using MTT Assay. A clinical isolate of MRSA was employed as a model for searching anti-bacterial agents. Healthy adult male Wistar rats that weighed 200 g were injected intravenously by MRSA used in this study to provide MRSA bacteremia model. Nanocurcumin was administered from intra peritoneal root.

Results: The synthesized nanocurcumin was completely soluble in water and was highly biocompatible and do not possess any significant toxicity in vitro on human fibroblast cells. The results revealed that the Nanocurcumin could inhibit the growth of MRSA. Furthermore, treatments with the nanocurcumin efficiently inhibited the bacteremia of the pathogen in rat model after 24h.

Conclusion: In conclusion our results clearly demonstrated that nanocurcumin could prevent the MRSA development and inhibit the death from bacteremia in rat model that was significantly comparable with imipenem and amikacin treatment.

Keywords: Bacteremia, MRSA, Nanocurcumin, Nanodrug

P190: **Isolation of a Methicillin resistant Staphylococcus aureus and its lytic bacteriophage from a same hospital in Tehran**

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2- Microbiology Department, Islamic azad University of Lahijan

Background and Aim: The emergence of antibiotic resistant bacterial strains requires the exploration of alternative antibacterial therapies. So we started to study the ability of bacterial viruses (bacteriophages) to lyses methicillin resistant Staphylococcus aureus (MRSA) colonies. Colonization of the MRSA in cardiac patients has become endemic in many hospitals and nursing homes in the Iran. Staphylococcal endocarditis and bacteremia are devastating infections associated with mortality rates of between 20% and 25% of affected individuals.

Methods: A strain of MRSA was isolated from a patient's blood sample with endocarditis in Imam Khomeini hospital. The phages were isolated from a municipal sewage treatment plant in Imam Khomeini hospital, too. The isolations performed by adding salt (58 g of NaCl) to 1 liter of sewage followed by centrifugation at 10,000□ g for 10 min. The supernatant was decanted into a separate container and mixed with polyethylene glycol (PEG) to provide a final concentration of PEG of 10% (wt/vol). The PEG containing supernatant was precipitated and the resulting precipitate was dissolved in 5 ml of phage dilution buffer (SM) and extracted once with an equal volume of chloroform. Phage plaques on MRSA cultures were harvested from the plate and single plaques were purified from a collection of phages that were isolated from sewage treatment plant.

Results: The phage strain obtained in this study had lytic activity against the clinical isolates of MRSA. This effect was comparable with streptomycine and clavulanic acid and remained after several passages on the same strain of MRSA.

Conclusion: This is the first report of isolation of MRSA bacteriophage from the hospital sewage in Tehran. The applications of this lytic phage as a potential for phage therapy could be considered as the significance of the present study.

Keywords: This is the first report of isolation of MRSA bacteriophage from the hospital sewage in Tehran. The applications of this lytic phage as a potential for phage therapy could be considered as the signific

P191: **Comparison of Susceptibility of Planktonic Cells and Biofilm of C.albicans ATCC10231 to Extract Myrtus communis**

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2- TarbiatModares University of Medical science, Tehran, IR of Iran

Background and Aim: Candida is a fungus that inhabits in a half of oral cavities of human. A main strong factor of Candida species is its ability to adhere to surfaces and form attached microbial communities known as biofilms. Biofilms are resistance to many antimicrobial drugs. The aim of this study was to evaluate antifungal effect of Myrtus communis against Planktonic cells and biofilm of C.albicans ATCC10231.

Methods: At the first, C.albicans was cultured on sabouraud dextrose agar. After 24 hours, suspension including 1×10^{6}cells/ml was prepared. Biofilm was formed in microtitre plate. The minimum inhibitory concentration (MICs) and minimum fungicidal concentration (MFC) of antibiotic was determined according to CLSI by serial microdilution method. Finally, antibiofilm activity of Myrtuscommunis extract was examined by MTT method.

Results: The extract of Myrtus communis had inhibitory effect against C.albicans. The quantity of MIC50 and MFC were 16/52 µg/ml and 31/25 µg/ml, respectively. Furthermore, the result indicated that this extract was capable to inhibit 59/3% biofilm structure of this fungal specie in 4000 µg/ml concentration.

Conclusion: It can be concluded that extract of Myrtus communis had antifungal and antibiofilm activity against C.albicans ATCC1023. This extractcan be used as a new product shows antibiofilm activity. Further investigation should be done to evaluate the effect of this extract against other candida species.

Keywords: C.albicans, biofilm, Myrtus communis

P192: **Mentha spicata essential oil against Listeria monocytogenes in Lighvan cheese**

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3- Department of Epidemiology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.

Background and Aim: Listeria monocytogenes is a food-borne pathogen that is widespread in the environments. There are several reports on the isolation of L. monocytogenes from many kinds of cheeses. The aim of this study was to evaluate the antibacterial effect of Mentha spicata essential oil on L. monocytogenes in traditional Lighvan cheese.

Methods: L. monocytogenes was therefore added to sheep's milk and Mentha spicata essential oil was subsequently mixed with the milk at concentrations of 0, 2 and 2.5 percent, respectively. After the production of Lighvan cheese, sampling was performed on the cheese over several days.

Results: Results of this study showed that Mentha spicata essential oil at all concentrations was effective against L. monocytogenes in comparison with the control group ($P<0.001$). There was no significant difference between 2% and 2.5%. The effect of Mentha spicata essential oil against L. monocytogenes at 14˚C was greater than at 4˚C. In addition, with an increase in salt water concentration, the anti-Listerial effect of Mentha spicata essential oil also increased.

Conclusion: According to the findings of this study, it can be concluded that Mentha spicata essential oil has a strong anti L. monocytogenes effect in Lighvan cheese and during this study, compared with the control group, these essential oils caused a significant reduction or complete elimination of the L. monocytogenes population in Lighvan cheese. In addition, Mentha spicata essential oil combined with environmental factors (temperature and salt water) increases the effectiveness of essential oils.

Keywords: Listeria monocytogenes, Mentha spicata, Essential oil, traditional Lighvan cheese.

P193: **Vancomycin susceptible Enterococcus faecium and Enterococcus faecalis carring vanA gene isolated from poultry meat samples**

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2- Dept of Bacteriology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran

Background and Aim: vanA genotype is considered as major importance that is predominant type of resistance to vancomycin and teicoplanin. It is assumed that presence of this gene indicates the resistance but sometimes phenotype is different from genotype. This study was aimed to detect and report the vanA within vancomycin susceptible E. faecium and E. faecalis isolated from poultry meat samples collected from Tehran poultry meat dealers.

Methods: One hundred poultry meat samples (70 chickens and 30 turkeys) were screened for enterococci contamination. The isolates were confirmed as E. faecium and E. faecalis by bacteriological and biochemical tests. They were further characterized by antibiotic susceptibility assay. The agar dilution method was used minimum inhibitory concentrations (MICs) determination. Finally, the vancomycin MIC ≥4 µg/ml was determined for testing vanA gene, using specific primer sets.

Results: Totally 18 E. faecium and 10 E. faecalis were isolated including 7 vancomycin-susceptible E. faecium and 6 vancomycin-susceptible E. faecalis. All VSEF strains were vanA carrier except one isolate from each of them.

Conclusion: Up to our best knowledge, this is the first report of the presence of vancomycin-susceptible vanA-positive E. faecium and E. faecalis in poultry meat samples that probably due to major deletions in the Tn1546 vanA operon.

Keywords: vancomycin susceptibility, vanA, Enterococcus faecium, Enterococcus faecalis, poultry meat, Tehran

P194: **Production of a biopolymer from a marine microorganism**

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Background and Aim: The aim of this study was to produce a biopolymer from a marine microorganism.

Methods: For this purpose, seawater was used as the basal medium and the effect of different factors on the yield production of expopolysaccharide (EPS) from Enterobacter cloacae was evaluated, individually.

Results: The EPS yield of 0.56% was obtained after 24 hours cultivation in the seawater, but the concentration of the biopolymer in the medium was decreased with increasing incubation time from 1 day to 3 days. Results showed that addition of sucrose or NaCl at different concentrations of 1, 2, 3 and 4% to the seawater was not effective on EPS yield. However, no production was observed in the brine medium (1, 2, 3 and 4% NaCl). It seems that oil or paraffin (0.1 to 2%) were better carbon sources as EPS yield was increased with increasing in oil or paraffin concentration in the seawater. pH changes from 6 to 8 did not have significant effect on the biopolymer production. Phosphate (potassium phosphate) and nitrogen (ammonium sulfate) sources had also no effect on the EPS yield.

Conclusion: The results of this study showed the possible potential future application of this bacterium.

Keywords: biopolymer, Enterobacter sp.

P195: **Evaluating the effect of different factors and their interactions on biopolymer production efficiency by Enterobacter sp.**

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Background and Aim: Evaluating the interaction of different factors affecting the production of exopolysaccharide (EPS) from Enterobacter sp.

Methods: Therefore, the interaction of different factors affecting the production of exopolysaccharide (EPS) from Enterobacter sp. cultivated in seawater as a basal medium for 24 hours at 30°C were investigated.

Results: Cultivation of Enterobacter sp. in the seawater as a basal medium resulted in 0.56% production of exopolysaccharide after 24 hours. Results revealed that biopolymer was not produced in formulated medium containing water, 0.05% nutrient broth, and 1% ammonium sulfate and K2HPO4, at different concentration of NaCl (1, 2, 3 and 4%). Addition of 1% paraffin as well as 0.05% phosphate potassium into 3.4% brine was effective; however, the yield was decreased to below a half. Diluting the seawater with distilled water at different ratios of 1: 2, 1: 1 and 2: 1 decreased the exopolysaccharide production yield. The reduction was more pronounced when the seawater was enriched with 1% glucose and 0.05% nutrient broth, under dilution condition. Enrichment of seawater with different concentrations of Aleo vera decreased the yield. Although addition of Aleo vera to 3.4% brine resulted in production of biopolymer in contrast to control, but the yield was very low (0.03%).

Conclusion: The efficiency of biopolymer production was not improved in the modeled mediums or by enrichment of seawater.

Keywords: Modeling, biopolymer, Enterobacter sp.

P196: **Survey of Aflatoxin M1 Contamination in Pasteurised Milk**

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Background and Aim: Survey of Aflatoxin M1 Contamination in Pasteurised Milk Fatemeh Shishehborzadeh1, Reza Kazemi darsanaki 2, Morteza Azizollahi Aliabadi 1 1. Department of Microbiology, Faculty of Basic Science, Lahijan Branch, Islamic Azad University, Lahijan, Iran 2. Young Researchers Club, Lahijan Branch, Islamic Azad University, Lahijan, Iran Abstract Aflatoxins (AF) are mycotoxins produced by certain fungi, especially Aspergillus flavus. Aflatoxin M1 (AFM1) is a hepatocarcinogen found in milk of animals that have consumed feeds contaminated with aflatoxin B1 (AFB1). These metabolites are not destroyed during the pasteurization and heating process. This study was undertaken to determine the presence and levels of aflatoxin M1 (AFM1) in pasteurised milk consumed in Guilan, Iran. A total of 40 samples were randomly obtained from retail outlets. Enzyme Linked Immuno Sorbent Assay (ELISA) technique was used to determine the presence and the level of AFM1. In 35 of the 40 milk samples examined (87.5%), the presence of AFM1 was detected in concentrations between 7.2ng/l and 98ng/l. AFM1 in 28 samples (70%) were higher than the maximum tolerance limit (50 ng/l) accepted by some of the European countries. The results of this study imply that more emphasis should be given to the routine AFM1 inspection of milk and dairy products in the Iran region. Furthermore, both farmers and dairy companies should be informed on the importance of AFM1, and the consequences of the presence of the aflatoxin in dairy products. Keywords: Aflatoxin M1, ELISA, Pasteurised Milk.

Methods: A total of 40 samples were randomly obtained from retail outlets. Enzyme Linked Immuno Sorbent Assay (ELISA) technique was used to determine the presence and the level of AFM1.

Results: In 35 of the 40 milk samples examined (87.5%), the presence of AFM1 was detected in concentrations between 7.2ng/l and 98ng/l. AFM1 in 28 samples (70%) were higher than the maximum tolerance limit (50 ng/l) accepted by some of the European countries.

Conclusion: The results of this study imply that more emphasis should be given to the routine AFM1 inspection of milk and dairy products in the Iran region. Furthermore, both farmers and dairy companies should be informed on the importance of AFM1, and the consequences of the presence of the aflatoxin in dairy products.

Keywords: Keywords: Aflatoxin M1, ELISA, Pasteurised Milk.

P197: **Investigation Antioxidant, In Vitro Antibacterial Activities Of Prickly Pear Cacti (Opunita) Extracts Against Food Spoiling Bacteria**

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Background and Aim: Prickly pear cacti have flattened, pad-like stems and Includes species in the genus opunita. Prickly pear fruits (PPF) constituted valuable foodstuff for animal and human. In this context, the present study aimed to investigation this fruit the antimicrobial activity against gram-positive bacteria and antioxidant activity.

Methods: prickly pear fruits were clean, washed with water and peeled. They were cut into small pieces with a clean knife. The Methanolic, Ethanolic and water extracts of PPFs were screened against Staphylococcus aureus , Bacillus cereus, Staphylococcus aureus resistant to methicilin (MRSA), Bacillus subtilis by well diffusion method. The samples were added to the wells at 25µl, 50µl, 75µl, 100µl concentration. The plates were then incubated at 37˚c for 24 hours. After 24 hours, the activity was measured by the presence of inhibitory zone and bacterial strains used in this study were collected from microbial laboratories, Iran. Also phenol and flavonoid content of the extracts were measured by folin ciocalteu and AlCl3 assays.DPPH was used for determination of free radical-scaveging activity of prickly pear fruit the extracts

Results: The results showed based software SPPS that the extracts of the prickly pear fruit have antimicrobial activity against food spoiling bacteria used in this study. Among the investigated extracts, the ethanol fractions exhibited highest antibacterial effect against Bacillus cereus, MRSA, whiles the methanol fractions exhibited highest antibacterial effect against Bacillus subtilis and staphylococcus aureus. Antioxidant average was 38.641±3.523mg/ml.the total obtained phenols was in terms of galic acid 641.266±9.668 mg/g and the flavonoid content of the extract in terms of quercetin equivalent was 128.462± 0.979mg/g.

Conclusion: This survey reports the anti-bacterial good activity of prickly pear, this means that there is in the opunita high degree of antibacterial activity which this effect may be due to the presence of potentially effective compounds in opunita

Keywords: Antibacterial activity, antioxidant activity, prickly pear cacti, spoiling bacteria, Opunita

P198: **Isolation of Listeria monocytogenes from milk used for Lighvan cheese production in Lighvan cheese factories**

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3- Department of Epidemiology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.

Background and Aim: Traditional Lighvan cheese is a semi-hard cheese, which has a popular market in Iran and neighboring countries. The aim of this study was to determine the status of contamination of milk used for Lighvan cheese production with Listeria monocytogenes.

Methods: This study was carried out between 2010-2011. Milk samples were randomly collected from milk tanks in various Lighvan cheese producing factories. Isolation of L. monocytogenes was performed according to ISO 11290 and biochemical tests were used to identify and confirm L. monocytogenes.

Results: 50% of collected samples from milk tanks in Lighvan cheese producing factories were contaminated with L. monocytogenes. The average level of L. monocytogenes contamination among positive milk samples was 40 CFU/ml.

Conclusion: This study is the first report of L. monocytogenes contamination in raw milk used for Lighvan cheese production in Iran. Due to fact that this cheese is produced from raw milk without any heat processing, the contamination of its milk can pose a potential risk for consumers.

Keywords: Listeria monocytogenes, raw milk, Lighvan cheese, Iran.

P199: **The Effect of Temperature on Antibacterial Activity of Lactobacillus spp. Derived Supernatant Against Proteus mirabilis**

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Background and Aim: Lactic acid bacteria (LAB) are generally recognized as safe (GRAS microorganisms) and play an important role in food and feed fermentation and preservation either as the natural microflora or as starter cultures added under controlled conditions. Proteus mirabilis, members of the family Enterobacteriaceae, are motile gram negative bacteria, that cause urinary tract infections. The aim of this study was to investigate the effects of temperature on antibacterial activity of Lactobacillus spp. derived supernatant against Proteus mirabilis growth

Methods: In this study the antimicrobial activity of five selected Lactobacillus spp. derived supernatant treated 0,10,30and 60 minute with 100 ˚c was tested against Proteus spps. growth by Agar Well Diffusion (AWD) assay.

Results: Results showed that the 100 ?c temperature conditons after 30 minute can reduce significantly the antimicrobial activity of lactobacillus spp. Against proteus mirabilis growth ($P < 0.05$).

Conclusion: it can be conclude that heating the supernatant fluids of lactobacillus spp. Can reduce the antimicrobial activity, trough damaging of antimicrobial compounds.

Keywords: Lactobacillus spp., temperature treating , Antimicrobial activity

P200: **A study of the frequency of entrotoxin genes A&B in coagulase-positive staphylococcus taken from confectionaries by PCR technique**

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4- PhD student in virology, Tarbiat Modares University, Tehran, Iran

Background and Aim: Confectionary contamination in the world is pretty widespread. The purpose of this study was to examine entrotoxin genes A&B in coagulase-positive staphylococcus in a sample of confectionaries in Khoramabad city

Methods: We selected 300 samples randomly and used the related standards of Organization of Standards and Industrial Research to test them for contamination. We used PCR technique to analyze entrotoxin genes A&B.

Results: 21 confectionary samples (7%) were separated from staphylococcus aureus and tested. The findings indicated that 20 samples of all confectionary samples (95.23%) were contaminated by SEA gene. No SEB gene was observed.

Conclusion: Entrotoxin Genes A & B in coagulase-positive staphylococcus are the main causes of food poisoning. Therefore, it is required to diagnose and control these causes. It is extremely critical to consider it as an necessary quality control standard in all confectionary workshops.

Keywords: Confectionary, staphylococcus aureus, entrotoxin genes A&B, coagulase-positive staphylococci, Khoramabad City

P201: **A study of fungal contaminations frequency in candies and pastries**

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5- an undergraduate of Health in the Azad University of Borojerd, Iran

Background and Aim: Confectionary products form an important part of foodstuffs in this country. The purpose of this study was to examine fungal contaminations, yeast and mould, in a selection of candies and pastries in Khoramabad city.

Methods: We selected 300 samples and used the related standards of Organization of Standards and Industrial Research to test them for contamination. We used IBM SPSS Statistics 19 to analyze the data.

Results: The findings indicated that 22% of all pastry samples were contaminated by mould and 10% and 30% of candy samples were contaminated by mould and yeast respectively. Rates of contamination were above average.

Conclusion: Therefore, it is required to act upon health and hygiene regulations through all processes of production to sales. It is absolutely vital to consider it as an obligatory quality control standard in all confectionary workshops.

Keywords: Fungal contamination, candies and pastries, yeast, mycology, Khoramabad City

P202: **Stability evaluation of macrolide antibiotic residues in meat during different cooking procedures**

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Background and Aim: The administration of veterinary antibiotics to food-producing animals may lead to the occurrence of residues in the edible tissues, milk, or eggs produced for human consumption. Antibiotic residues in food had made different concerns, such as Alteration of intestinal microflora and the possible development of resistant strains which cause failure of antibiotic therapy in clinical situations. Macrolide antibiotic residue, for example tylosin, has great importance from the public health point of view because they were widely consumed in veterinary medicine. Residue levels in food products such as meat may change during cooking. Therefore, cooking influences the level of risk posed by such residues. The goal of this research is to determine the effect of boiling and frying procedure on tylosin residues in chicken muscle.

Methods: Minced chicken muscle samples containing various tylosin levels (200, 400 and 800 µg/kg) were cooked by procedures of boiling at 100 0C (10, 20 and 30 min) and frying at 180 0C (3, 5 and 7 min). Tylosin residue extraction was carried out using cation-exchange cartridges. Tylosin amount was measured before and after cooking by reversed-phase high performance liquid chromatography (HPLC) with UV detection at 289 nm.

Results: In all treatments, tylosin levels in cooked samples were significantly less than that of raw samples ($p< 0.05$). By increasing boiling and frying time, more tylosin amount lost. Between tylosin reduction percentage of samples contained various initial concentrations of tylosin (200, 400 and 800 ?g/kg) and boiled or fried, significant difference was observed. In frying procedure, tylosin loss depended on initial level in raw meat (200, 400 and 800 ?g/kg) and when tylosin level in raw meat was increased from 200 to 800 ?g/kg, change amount observed was less. In general, boiling procedure of chicken muscle tissue for 30 min resulted in a 86.9% decrease of tylosin residues, whereas its frying for 7 min resulted in a 62.9% decrease of the antibiotic concentration.

Conclusion: Tylosin residue during meat cooking decreases and its loss depends on type of cooking procedure, heating time and temperature and the initial concentration of tylosin. In summary, Surveillance data obtained for tylosin residue levels in raw food products aren't directly applicable for dietary intake calculations when the whole cooked product is consumed. Exposure to residues of tylosin antibiotic may be reduced by suitable cooking.

Keywords: Antibiotic resistance, drug residue, macrolide, food processing, food safety

P204: **Antimicrobial activity of microencapsulated Satureja hortensis essential oil (SEO) against two pathogenic bacteria**

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Background and Aim: Essential oils (Eos) are oily, volatile and natural compounds obtained from plants. In recent years they are broadly used as antioxidant and antimicrobial agents in food and pharmaceutical industries. Satureja essential oil (SEO) extracted from Satureja hortensis is used as seasoning agent and traditional herb in Iran. Antimicrobial and antioxidant activity of SEO have been demonstrated. However like other EOs, SEO is volatile and sensitive to environmental condition. In order to improve its stability and bioavailability, SEO has been encapsulated in alginate microcapsules and its antimicrobial activity against Staphylococcus aureus, and Salmonella typhimurium was investigated.

Methods: In order to preparation of microencapsulated SEO, an emulsification-ionic gelatin method was used with three concentration of this essential oil (0 (control), 1, 2, and 3 % v/v). Antimicrobial activity of samples was assessed using the agar well diffusion assay. Three wells of 6 mm diameter were punched into the agar on each plate (that seeded by 100µl of an overnight broth culture containing 107-108 CFU/ml of the test organisms) and lyophilised microcapsules were loaded into wells. Plates were incubated at 30 ºC for 24 h. The whole zone area was calculated then subtracted from the well area and this difference in area was expressed as the zone of inhibition (mm2). All measurements were performed triplicate.

Results: The results of this study indicated that there was no inhibition zone around unloaded microcapsuls (control) in two tested microorganisms. Microcapsules containing 1% v/v SEO exhibited antibacterial activities with zone of inhibition 28 mm2 against S. aureus, but could not inhibit S. typhimurium. With increasing SEO concentration inhibition zone increased significantly. Alginate microcapsule with high SEO content (3% v/v) showed maximum inhibition zone for both studied bacteria. SEO-loaded microcapsules showed the more antimicrobial activity against S. aureus (with an inhibition zone 302 mm2) than to S. typhimurium. It was noted that essential oils are more active against gram positive than gram negative bacteria. This may be due to single membrane glycoprotein/techoic acid of Gram-positive bacteria as compared with double membrane of gram negative bacteria. Moreover difference in hydrophobicity of cell surface is another reason for this observation. The antimicrobial activity of essential oils can be explained by the presence antimicrobial compounds. It has been reported that carvacrol , γ-terpinene and p-cymene are the most components in Iranian SEO. These lipophylic compounds interact with phospholipids present in cell wall of bacteria and cause increased membrane fluidity and leakage of cytoplasm.

Conclusion: Our findings demonstrated that alginate microcapsules incorporating SEO have a good potential to be used as an antimicrobial agent in the food and nutraceuticals industries. Also further studies are needed to test the effectiveness of these antimicrobial microcapsules on food matrixes.

Keywords: essential oils, antimicrobial activity, encapsulation.

P205: **Evaluation of antibiotic susceptibility pattern and resistance gene in indigenous lactic acid strains with potential probiotic and starter properties.**

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Background and Aim: The antibiotic susceptibility patterns of some species of lactic acid bacteria isolated from traditional dairy products made in different geographic regions of Iran have been evaluated by analyzing their isolated strains used for production of probiotics.

Methods: Antimicrobial susceptibility of 45 screened isolates was tested by 10 Antibiotic groups with disc diffusion and MIC method after species-level identification. Resistant strains were selected and examined for the presence of resistance genes by PCR.

Results: It was found that antibiotic resistance lactic acid bacteria are widespread among Iranian traditional dairy products and resistance incidences depended on the raw material and manufacturing area of the food. Distribution of resistance was found in different species. Isolates were susceptible to chloramphenicol, tetracycline, ampicillin , cephalothin, and clindamycin. In contrast, most strains were resistant to vancomycin, rifampicin, kanamycin, ciprofloxacin, trimethoprim/sulphamethoxazole ,ciprofloxacin and metronidazole. Intrinsic aminoglycosides ,quinolones ,sulfonamides and metronidazole resistance were observed in all strains. The vanX , vanA ,erm(B), tet(M) genes and msr were detected on either plasmid or chromosomal DNA of certain LAB isolates. For chloramphenicol,β-lactames and cephalosporins no resistance was detected.

Conclusion: Antibiotic resistance is present in different species of probiotic strains, which poses a threat to food safety. Evaluation of the safety of lactic acid bacteria for human consumption should be guided by established criteria, guidelines and regulations.

Keywords: Antimicrobial susceptibility; Lactic acid bacteria; Antibiotic resistance genes

P206: **Prevalence of Escherichia coli Enter hemorrhagic in traditional cream of milk from cows of rural communities in the city of Marand**

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Background and Aim:: E. coli bacteria (E.coli) usually live in the human gastrointestinal tract and are not pathogenic. However, certain strains of this bacterium under certain conditions can cause various diseases the bacterial contamination rate in the last decade has doubled annually and each year more than 17 thousand cases of infection in hospitals are reported. Gastrointestinal infection is caused by certain strains depend on type of strain varied from simple diarrhea to diarrhea with blood. Escherichia coli enter hemorrhagic (EHEC) causes the bloody diarrhea syndrome in patients. Disease of cattle and calves, handling meat, Daley products, and tools, can play role of pollution source in disease transmission. Non-pasteurized milk, inadequate cooking meat (eg. hamburgers) also can be one of tile reasons is the prevalence of this disease. The purpose of this study was to evaluate the prevalence of disease and investigate antibiotic susceptibility of strain isolates from non-pasteurized cream from cows of 3 villages of Marand, Abraghan, Bangin and Barooj.

Methods: In this study, 500 samples of cream were collected from three villages of Marand in 1389 (2010). The samples cultured after enrichment in T58, BHI and ECB with novobiocin and were evaluated. Finally, using specific isolation antiserum of tile bacteria 0157 H7, E.coli was confirmed and were investigated by standard susceptibility methods to seven different antibiotics.

Results: In this study the most appropriate method of enrichment was determined using T5B medium and the total sample of 45 sorbitol-negative E. coli were isolation. Also E. coli detection rate of 3.40% was acquired, and all they resist to penicillin and novobiocin.

Conclusion:: Due to the pathogenicity and the low Infectious dose of bacteria E.coli 0157: H7, larger studies are recommended on other dairy products in the country.

Keywords: Escherichia coli enterohemorrhagic, cream ,pathogenicity

P207: **Effects C on the Microbiological Changes of Brushtooth°18–of Frozen Storage at Lizardfish (Saurida undosquamis) Burgers With and Without Coating**

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Background and Aim: Fish burgers are produced as battered and breaded products and are commonly stored and sold in frozen state. Frozen storage is one of the most important techniques for preserving fish products. Bacterial growth is the main cause of fish spoilage, and so it is logical to use bacterial counts as an index of quality.Brushtooth lizardfish (Saurida undosquamis), distributed in the Persian Gulf and in the sea of Oman, are an important resource in the far east, and annually tons of this species is eaten mainly in the form of fish cakes. Thus, the objective of this research was to produce fish burger from deep flounder with and without coating and to evaluate the microbialattributes during storage C for 5 months.°18–at

Methods: For all microbiological counts, 10 g of sample was transferred into 90 ml NaCl solution (9 g Nacl/1000 ml distilled water) (ICMSF, 1978). From the 1/10 dilution, other decimal dilutions were prepared. Total plate count (TPC) and total coliform count (TCC) were determined by pour plate method using plate count agar, according to ISO 8443 (2003), and violet red bile agar, according to ISO 4832 (1991), as medium, respectively. Staphylococcus aureus and psychrotrophic bacteria were determined by surface plate method using Baird Parker agar, according to ICMSF (1978), and total plate count agar, according to ISO 17410 (2001), as medium, respectively. Escherichia coli were determined by most probable number method using lauryl sulfate broth as medium. Confirmation test was carried out using peptone water and EC broth according to ISO 7251 (2005).

Results: all microbiological counts decreased by C and this decrease was higher in Group B than that in°18–freezing at Group A, and Group B had lower TPC and TCC at the end of the storage period. Because of the antimicrobial effects of the additive ingredients in both of the groups and cooking temperature in Group A fish burgers, microbial load of fish burgers was lower than that of fish paste

Conclusion: combination of sensory evaluation and microbiological count, especially TPC, was more informative than the chemical analysis. This study showed that coating materials are favorable for brushtooth lizardfish, but batter and breading materials have no effect on development of shelf life in brushtooth lizardfish burgers

Keywords: Brushtooth Lizardfish (Saurida undosquamis), Fish burger, Coating, Frozen storage, Microbial change

P208: **Impact of different concentrations of sodium chloride on the growth behavior of Staphylococcus aureus in salted silver carp (Hypophthalmichthys molitrix) fillets**

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Background and Aim: Staphylococcus aureus (S. aureus) is one of the important foodborne bacteria in salted marine products. Salting is a traditional method for preservation which reduces corruption and increases shelf life. In Iran (especially in Northern provinces), consuming semi-cooked or raw salted fish is well recognized. Due to the presence of halophilic and salt-resistant microorganisms (such as S. aureus) in these products, occurrence of foodborne infection and intoxication is probable. The purpose of this study was to evaluate the antimicrobial activity of different concentrations of NaCl on growth behavior of S. aureus in salted silver carp at unfavorable refrigeration storage temperature of 10°C.

Methods: The inoculums of S. aureus American Type Culture Collection (ATCC) 6538 obtained from the Department of Microbiology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran, was used in this study. Silver carp fillets (25 g) were exposed to gamma irradiation (5 KGy, using cobalt irradiator), to eliminate any likely background of S. aureus. Afterwards, fish fillets were marinated in different concentrations of sodium chloride (4%, 8% and 12%) (w/v) in the sterile screw capped flasks. A number of 12 fillets were placed in each flask. The flasks were placed in a refrigerator at 5ºC for 24 h. Subsequently, silver carp fillets were transferred into the new empty sterile bags and each fillet was inoculated with 1×103cfu/g in 10 points of its surface. The stomacher bags were placed in an incubator at unfavorable refrigeration storage temperature of 10ºC. To obtain the limit of toxicity dose of S. aureus (>106cfu/g), the bacteria were cultured from each treatment immediately at post-inoculation and on days 1, 2, 3, 6, 9, 12, 15, 18, 19 and 21.

Results: Different concentrations of NaCl had significant inhibitory impact on the growth of S. aureus in salted fish fillets compared to control group (p<0/05). The control group reached to the maximum bacterial growth (>106cfu/g) after three days and subsequently, until day 21 had no significant difference. The fillets with different concentrations of NaCl (4%, 8% and 12%) (w/v) reached to the maximum S. aureus growth 6, 9 and 15 days post-storage, respectively.

Conclusion: This study indicated that application of NaCl without any complementary element is not considered as a good preservative for extending the shelf life of salted silver carp. However, salting reduced corruption and bacterial growth. Since, this preservation method prevents the alteration of fats and proteins of fillets. The World Health Organization (WHO) has recently called for a worldwide reduction in the consumption of salt in order to decrease the incidence of cardiovascular disease (CVD). To diminish the level of salt in the processed foods and maintain the safety of products, using natural preservatives such as essential oils of plants are the best choice.

Keywords: Staphylococcus aureus, Bacterial growth, Sodium chloride, Salting, Silver carp

P209: **Prevalence of parasitic contamination in vegetables used for raw consumption in Rasht, Iran: Influence of season and washing procedure**

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Background and Aim: This study aimed to determine the prevalence of intestinal parasites in raw vegetables consumed in rasht, Iran; and efficacy of season and washing procedures to remove parasites from the vegetables. A total of 120 field vegetable samples composed of parsley, spearmint, scallion, basil, coriander, lettuce, cress, leek, tarragon, and radish were purchased from the vegetable markets. Each sample was divided into three groups. One group was used as the unwashed sample and the second and third groups were washed with traditional and standard washing procedures, respectively.

Methods: A total of 120 field vegetable samples were purchased from 5 retail vegetable markets in rasht, Iran during February 2011 to January 2012. The vegetable samples were transported to laboratory in plastic bags. In traditional procedure which is generally used for washing of vegetables in Iran, the vegetables are immersed in tap water inside a sink. It is left (approx. 10 min) for sedimentation of mud and dust. Then, it is gently collected, put in a plastic basket, and rinsed for 5 min with tap water. In the standard procedure described by Bier (1991), the vegetables are washed with tap water to remove mud and dust, disinfected by immersion for 30 min in a solution containing 200 ppm of active calcium hypochlorite, and finally rinsed in an automated fruit-vegetable washer (EASTECH, model SXQ8-BA, Guangdong, China) for 10 min. The unwashed and washed groups of vegetable samples were examined for the presence of parasites. The preparation was examined under a light microscope for detection of parasites (eggs, cysts and larva) using _10 and _40objectives.

Results: Intestinal parasites were detected in 52.7% of unwashed, 3.6% of traditionally washed and not in any standard washed samples ($P < 0.001$). The parasites were detected in unwashed vegetable samples included Ascaris lumbricoides eggs (16.2%), Taeniid spp. eggs (8.6%), Toxocara spp. eggs (4.1%), Trichostrongylus spp. eggs (2.4%), Fasciola spp. Eggs (18.2%) Giardia spp. cysts (11.3%) and non-pathogenic protozoan Entamoeba coli cysts (7.4%). The rate of parasitic contamination in different seasons was found to be 49.7% in spring, 38.4% in summer, 12.4% in autumn and 32.6% in winter ($P < 0.005$).

Conclusion: The results of the present study emphasize the importance of raw vegetables in the transmission of intestinal parasites to human. It is necessary to improve the sanitary conditions in the areas where the vegetables are cultivated and consumed. The local health and environmental authorities should train the consumers on the potential health consequences of the intestinal parasites through consumption of raw vegetables, and the importance of proper washing and disinfecting of vegetables before consumption. In addition, the proper treatment of urban or rural wastewater used for irrigation of vegetables should be implemented.

Keywords: Raw vegetables , washing procedures, parasitic contamination, Rasht

P210: **Modelling the effects of Carum copticum essential oil, pH, inoculum level and temperature on staphylococcus aureus**

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Background and Aim: Staphylococcus aureus is one of the most important pathogenic bacteria which is the second cause of foodborne diseases after salmonella spp. (Akineden et al. 2008). The genus staphylococcus is member of the staphylococcaceae family; they are gram-positive, non-motile, nonsporulated, facultative anaerobic, catalase positive and resistant to adverse environmental conditions. They are halotolerant, therefore most of them can grow in media with 10% of NaCl (Vasconcelos et al., 2010). S. aureus can cause a form of gastroenteritis which is resulted from the ingestion of one or more staphylococcal enterotoxins contained in food that has been contaminated with these bacteria. Many intrinsic and extrinsic factors (physicochemical factors) affect the growth of foodborne microbial pathogens. Therefore in order to control and limit their potential risks, several studies have focused their investigations on environmental conditions which affect the growth of pathogens (McCann et al., 2003). Predictive microbiology can predict the responses of population of microorganisms to particular environmental conditions such as temperature, pH and aw with the help of mathematical models (built with data from laboratory testing) and computer software to graphically describe these responses. Predictive modeling has been applied in food microbiology to describe the growth behavior of specific pathogens (Jamshidi et al., 2008). Due to the increasing knowledge of the adverse effects of using chemical preservatives, biopreservatives and natural antimicrobial compounds become in the point of interest of both scientifics and ordinary people (Burt et al., 2004).

Methods: This study was designed to examine the combined effects of different concentrations of Carum copticum (Zenyan in Persian) essential oil (0%, 0.015%, 0.03%, 0.045%), two incubation temperatures (35 ˚C, 25 ˚C), three levels of pH (5, 6, 7) and two inoculum size (103, 105 cfu/ml) on the growth of staphylococcus aureus in brain heart infusion broth. The experiment was carried out in triplicate. Growth was monitored by visible turbidity during a 30-day period. To evaluate effects of explanatory variable on time to detection (TTD) of bacterial growth, parametric survival models based on the weibull distribution was used.

Results: All explanatory variables had significant association with time to detection ($P<0.05$). The final model accurately predicted the growth initiation and inhibition of Staphylococcus aureus.

Conclusion: These models could predict the most efficient combinations of factors to stop microbial growth, so giving a significant degree of safety from spoilage or foodborne disease. Another important criteria in this method of preventing microbial growth, is the validation of the experimental data with the data collected from truly conditions that foods are stored by customers

Keywords: Staphylococcus aureus, Carum copticum essential oil, Modeling, Predictive microbiology

P211: **Determining the Presence of Enterotoxin Encoding Genes A to E in Staphylococcus Aureus Isolated from Cheese**

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Background and Aim: Staphylococcus aureus causes food poisoning through releasing enterotoxin in food. This study was conducted with the aim of evaluating the presence of enterotoxin-producing genes A to E and antibiotic resistance in coagulase-positive Staphylococcus aureus isolated from cheese samples offered in Mashhad, Iran.

Methods: Staphylococcus was isolated from 25 samples (25%) out of 100 samples of the studied cheese. All isolated samples were confirmed through PCR method.

Results: Among a total of the confirmed 25 strains of Staphylococcus aureus, 6 strains (24%) had at least one enterotoxin encoding gene. The highest frequency was related to the 4 strains enterotoxin-producing gene A (sea), (16%) and then 2 strains enterotoxin-producing gene C (sec), (8%). None of the strains had enterotoxin-producing genes E, D, B.

Conclusion: So, it is of particular importance to control food stuff for possible Staphylococcus aureus and enterotoxins produced by regulatory institutions. Therefore, it is suggested that given culture and consumption level in community of any country, the monitoring and supervisory control system of these strains or reducing the amount of staphylococcal food poisoning be independently performed.

Keywords: Staphylococcus aureus, enterotoxin, PCR

P212: **Detection of toxin profile in Clostridium perfringens isolated from Poultry products using culture and PCR methods**

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Background and Aim: Clostridium perfringens is an important pathogen among humans and many other animal species. Clostridium perfringens has been identified as agent of many enteric diseases, including necrotic and hemorrhagic enteritis in animals, as well as food poisoning and sporadic diarrhea in humans. C. perfringens isolates are categorized into five toxin types: A, B, C, D and E, depending on the production of four major toxins: alpha, beta, epsilon and iota.

Methods: In a cross sectional study, 180 samples of fresh poultry products including neck, wing and liver (each 50 samples) and 30 samples of gizzard were collected randomly from retail stores in Mashhad. In this study, C. perfringens isolated by cultural methods and confirmed by PCR methods. Toxin profile of isolates were confirmed by Multiplex PCR.

Results: Out of 180 samples, 6 samples were positive in culture methods. The Cpa and cpb genes were found in approximately 3.33% of samples. All of the isolates confirmed as C. perfringens type C by PCR. In Single PCR for 6 isolates of Clostridium perfringens?The netB gene was identified in 5 isolates (83.33%) and the tpeL gene was only identified in 3 isolates (50%).

Conclusion: We can conclude that multiplex PCR may provide a useful and reliable tool for C. perfringens genotyping in routine veterinary diagnostics.

Keywords: Clostridium perfringens ،Multiplex PCR،cpa،cpb

P213: **Effect of , Lactobacillus(Y2F3)- a native probiotic- on passive avoidance learning of male Wistar rats under chronic stress**

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Background and Aim: The aim of this study was examination of Y2F3, the native Iranian probiotic isolated from Traditional dairy product effect on the rat passive avoidance.

Methods: In this study 24 male wistar rats with 95-118 grams weight range and 4 weeks age were used and they divided to four groups randomly: two groups as control, with and without stressed rats. Two other groups were received were gavaged with 1 * 10 9 cfu/ml of probiotics.

Results: Data of this research indicated that in the control groups, stress were increased the time of latency in dark component significantly ($p<0.05$) and thereby decreased the learning and increased the spent time in dark component. In probiotic receiving groups, learning level had not significant difference with non-stressed control group even in stressed condition.

Conclusion: results of this study showed that the lactobacillus(Y2F3) isolated from Tarkhineh dough, enhance the passive avoidance learning of male Wistar rats under stress conditions. Our data demonsrtrate the positive effect of this probiotic on passive avoidance learning

Keywords: passive avoidance learning, probiotic, stress

P214: **Evaluation of drug-resistant pattern in Salmonella strains isolated from native eggs in Yasouj , Iran**

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Background and Aim: Food-borne diseases are a major health problem among industrial and non-industrial economic and salmonellosis is the most common form of food poisoning in Worldwide, also Salmonella can cause mild gastroenteritis to fatal septicemia in humans. Adding antibiotics to the diet can cause drug resistance in Salmonella in domestic animals which can be transmitted to humans through food, The aim of this study is to investigate the prevalence of Salmonella strains isolated from native eggs in Yasouj city and Antimicrobial Resistance of them.

Methods: This study was conducted as a cross - sectional on the collection of 210 native eggs collected in different areas of the Yasouj city, during the months of June to August 2012, to study the pattern of drug-resistant bacteria and salmonella contamination. Sampling was carried out from the shell, and yolks egg separately and after enrichment in F selenite broth, for the growth and isolation In SS (Salmonella - Shigella) Medium were cultured. Salmonella suspect colonies were assessed using tests TSI, SIM, MR-VP, urease and lysine decarboxylase, Then the samples were confirmed by PCR using invA specific primer.

Results:: After performing biochemical tests and PCR, it was found that 14 number of eggs (6/66%) were contaminated with Salmonella. With investigated of antibiotic resistance pattern of Salmonella, One hundred percent of the strains were sensitive to the kanamycin, streptomycin, trimethoprim and ciprofloxacin antibiotics. Besides the78/57% of samples to cephalexin and 71/42% of samples to tetracycline were sensitive. Most antibiotic resistance to penicillin (100%) and neomycin (78/57) was observed. Also Samples were Half-sensitive to the neomycin (21/42%), tetracycline (28/57%) and Cephalexin (21/42%) antibiotics.

Conclusion: Microbial agents can cause food spoilage and disease, so it is highly important nutrients for microbial control. To prevent the build up of resistance in different Salmonella serotypes recommended antibiotic overuse in livestock and poultry should be avoided. Complete resistance to penicillin and lack of perfect sensitivity to cephalexin, tetracycline and neomycin can result in overuse of antibiotics in the treatment of cases of resistance to them is not unexpected.

Keywords: salmonella, antibiotic resistance, PCR

P215: **The effect of Iranian native fermented milk as a probiotic on cardiovascular disease risk factors in male Wistar rats model under stress**

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Background and Aim: Chronic stress and high levels of plasma cholesterol are two important risk factors for cardiovascular disease. It is shown that a 1% reduction in cholesterol, 2 to 3% reduces the incidence of cardiovascular disease. To reduce blood cholesterol can be used in many different ways, such as drug therapy or the use of probiotics. The purpose of this study was to evaluate the effect of Iranian native fermented milk as a probiotic on risk factors for cardiovascular disease in male Wistar rats model under stress. This fermented milk is a new probiotics product which produced by Iranian native strains.

Methods:: For this study 42 male Wistar rats with the same age, were randomly grouped. Mice were followed for 21 days: two control groups which received regular and fat food respectively without stress, Fatty control stress, 3 groups were feed with highly fated food with probiotics (1ml), lovastatin (10 mg/kg) and probiotics along with lovastatin respectively under stress and a probiotics group without stress. The used probiotic was fermented drink milk had 5 Lactobacillus strains (5× 106cfu/ml) which were isolated from Iranian indigenous fermented dairy products. After bleeding, was analayzed serum by BT3000 autoanalyser set & diagnostic kits Pars Azmoon, Blood glucose, was determined level of cholesterol, HDL, LDL, CPK.

Results: Results analysis by prism software and T-tests and analysis of variance. Cholesterol levels were significantly increased with consumption of fatty foods. , significantly decreased ($p<0.05$) the blood cholesterol levels from164.3± 13.63 to 102.5 ±7.932 and 93 ±7.45 mg/dl, respectively. There was no synergy between drug and probiotics. Significant increase in blood glucose was observed in the Stress control group compared to other groups. CPK level no significantly in the stress control group compared to 3 tried group under stress.

Conclusion: based on these results it seems that Iranian native fermented milk such as the drug can help prevent cardiovascular disease.

Keywords: fermented milk, probiotic, cardiovascular, Chronic stress, cholesterol

P216: **A Study of the Microbial Load of Fruit Rolls using Total Count at District 02,Tehran**

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Background and Aim: Introduction: Rolled fruit is a junk food favored by a wide range of age groups specially children and is made available in a variety of products appealing to people with diverse tastes. Therefore, failure to observe hygiene in production and ways to preserve the product from microbial contamination would have harmful effects on the consumer's good health. The product is still produced traditionally and offered in most recreational areas. Frequently the production is not hygienic. In this study, the degree of the microbial contamination of the entire range of the product was explored in an attempt to find a way to minimize the microbial load.

Methods: In this study 50 samples, repeated three times, of the entire range of rolled fruit available at District 02, Tehran were taken. Cultured for 48 hours at an incubator temperature of 37 degrees C under general culture conditions, "Nutrient Agar", the samples were subjected to colony counts.

Results: The findings indicated that out of the 150 plates prepared, 91.5% of them showed colony growth. In view of the elevated microbial load, it seems that a major problem in health and safety is food.

Conclusion: The findings indicated that out of the 150 plates prepared, 91.5% of them showed colony growth. In view of the elevated microbial load, it seems that a major problem in health and safety is food.

Keywords: Microbial Load, Rolled Fruit, Total Count

P217: **Reported four years Food-borne botulism (classic) in Iran**

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Background and Aim: Background: Botulism food poisoning is the most dangerous of food poisoning is caused by the Gram-positive ,obligate anaerobic , rod-shaped and spore-former , called Clostridium botulinum and it cause symptoms of flaccid paralysis to death by a neurotoxin produced. C. botulinum produces 7 immunologically distinct toxins, which are designated by the letters A–G.The incidence of toxicity in different countries be varies. Aim: The aim of this study was to find the most common causes Food-borne botulism and geographical distribution of the toxicosis in the interval of time since will be in the 2009 -2012 in Iran.

Methods: Methods: In this cross-sectional study , data on the incidence of botulism food poisoning According to different provinces , food poisoning , and types of C. botulinum's toxins common collected in the years 2009 – 2012 and analysis of epidemiological survey conducted.

Results: Results: We studied 53 cases of botulism have been approved , of typeE (32.2%) , B (30.18%) and A (18.86%) as the most common toxin types are identified and reported. Salted fish and spawn with 49.01% of the most common foods that were cause botulism. Botulism food poisoning caused by the ingestion of autochthonal cheese 5.66% , buttermilk 3.77% , and tuna , olives , pickles , autochthonal yogurtandcurd , each with a total of 9.4% of cases reported have been recorded. Provinces of Golestan , Gilan ,West-Azerbaijan have the largest devoted to botulism poisoning.

Conclusion: Conclusion: Considering the high rate of food-borne botulism in the need to promote public awareness for the prevention of this type of poisoning may feel. Smoked and salted-fish should be thoroughly cooked before consumption. The spawn should be avoided eating raw and the spawn must complete steps to finish cooking. boil Cans before use for 20 minutes. Create anaerobic conditions (buried) in the preparation of autochthonal cheese and pitcher cheese must be removed. Add the oil in the production process of canned-house to prevent mold-cans should be avoided. Infants aged less than one years old and especially under six months old should not be fed with honey.

Keywords: Food-borne botulism , classic botulism ,Clostridium botulinum, Iran

P218: **Microbiological quality of cream sweets collected from Tehran south areas -summer 1391**

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Background and Aim: A variety of sweets especially cream sweets are more consumption confectionary products in our country. Due to this fact and more probability to microbial contamination, this study was accomplished to control microbial quality of fresh cream sweets in warmest season of the year in the southern areas of Tehran.

Methods: this study was fulfilled based on the national standard (NO. 2395 – 1387) on 60 samples collected from confectionaries in southern of Tehran.

Results: Findings of the survey showed 52 (86/66%) of the total samples aren't appropriate to use , 24(40%) have Entrobacteriacea count more than standard limit , 10 (16%) have E.coli , 14 (23/33%) have S . aureus and 30 samples have yeast count over the standard limit is mentioned.

Conclusion: Results indicated that the health status of production and distribution of this food – filled and sensitive and raw materials used especially cream don't have good quality , and since various reports claim of inappropriate health quality of sweets , more supervisions and more serious of health authorities are required to improve the safety of foods.

Keywords: Microbiological quality , Fresh – cream sweets , south of Tehran

P219: **Evaluation of novel and wide Immunoinformatics analysis progresses for Food Allergenic proteins**

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Background and Aim: Different types of food, pollen or dust mites are common sources of allergens. Food allergies is becoming an increasingly serious problem, especially in developing countries. The number of resources containing data on the sequence, structure, and evolution of allergenic proteins has increased significantly in the last several years. Simultaneously, The application of bioinformatics methods in immunology is a “hot topic” and immunoinformatics resources related to the prediction and functioning of the immune system continue to mature rapidly.

Methods: The ability to predict allergens becomes important these days because of the introduction of genetically modified foods and new modified proteins aimed at therapeutic usage. For example, Some web server can predicts the potential allergenicity of proteins and our query protein will be compared against a set of prebuilt allergenic motifs that have been obtained from known allergen proteins. The query will also be compared with known allergens that do not have detectable allergenic motifs. The key step to a protein specific allergic reaction is the binding of IgE antibodies to an allergen. Here the immune system recognizes peptide units within allergens. We introduce a prediction server that extracts protein motifs that are important for immune responses from known allergens. The server then compares user’s query proteins with these motifs to determine the potential allergenicity.

Results: Allergy is a world-wide problem. The official bioinformatics criteria recommended by WHO for the identification of proteins as allergens able to cross-react with previously known allergens are as follows: the presence of a common fragment containing at least 6–8 amino acid residues or the presence of a fragment containing at least 80 amino acid residues with an identity of at least 35%.

Conclusion: Methods have been developed to predict probable allergenicity from protein sequence, although more works need to be done for better and more precise prediction. In the present article, we’ll discuss recent applications of bioinformatics tools that shaped our current understanding about allergenicity of proteins.

Keywords: Allergenic proteins, Immunoinformatics, Applications, prediction

P220: **Antibacterial activity of Nisin against of some food-borne bacteria in fillets of rainbow trout (Oncorhynchus mykiss)**

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Background and Aim: Nisin is a natural antimicrobial peptide produced by strains of Lactococcus lactis subsp. Nisin is a well-known broad spectrum bacteriocin active against Gram-positive pathogens associated with foods.

Methods: Nisin was purchased from Sigma–Aldrich Chemical Co. (N5764; Sigma, St. Louis, Missouri, USA) that contains 2.5% nisin with minimum potency of 106 U/g. This study was undertaken to evaluate the effect of nisin (0, 0.5 and 0.75 µg/ml) against Streptococcus iniae, Lactococcus garvieae and Staphylococcus aureus in fillets of rainbow trout in 4ºc.

Results: The growth of S.aureus was significantly ($P<0.05$) decreased by nisin concentrations, but for S.iniae and L.garvieae the viable count was no significantly ($P>0.05$) inhibited by nisin concentrations (0.5 and 0.75 µg/ml) at 4º c.

Conclusion: Results of this study indicate that the nisin might be a useful candidate for using in food industry to control the growth of pathogenic microorganisms.

Keywords: Nisin, food-borne Bacterial, Oncorhynchus mykiss

P221: **Effect of Microencapsulation on Survival of Lactobacillus plantarum PTCC 1058 in Simulated Gastrointestinal Conditions**

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Background and Aim: The main purpose of this study was to examine survivability of free and microencapsulated Lactobacillus plantarum PTCC 1058 with alginate and resistant starch in simulated gastrointestinal conditions.

Methods: Mixtures of sodium alginate (2% w/v) and resistant starch (2% w/v) were prepared and pure bacterial mass (1% v/v) was added into the mixture. Then microcapsules were prepared by the use of emulsion method. Mean Size of beads was 19.87 ± 1.49 µm obtained by the Scan Electronic Microscope (SEM). The survivability of free and encapsulated cells of L. plantarum was examined in (0.6% w/v) bile salt solution (pH 8.25), simulated gastric juice (pH 1.55) and simulated intestinal juice with and without (0.6% w/v) bile salt (pH 7.43) by incubation at 37˚ C with 50 rpm mechanical tension for 120 min. Survivability was investigated every 30 min by pour plate culture in MRS agar and incubation at 37˚ C for 72 h. All of the stages carried out with triplicate trials.

Results: It is indicated that encapsulated L. plantarum could reach to the colon by the viable count of 6.48 and 7.23 log CFU/mL live cells, respectively at the presence and absence of bile salts in the intestinal juice, whereas, free bacteria could reach to the colon by 4.6 log CFU/mL live cells which is not sufficient for its probiotic function. Statistical analyses with ANOVA and Independent T-test showed that the survivability of encapsulated bacteria was significantly higher than free bacteria ($P<0.05$).

Conclusion: It is concluded that microencapsulation of L. plantarum with calcium alginate and resistant starch can effectively protect these bacteria against gastrointestinal adverse conditions.

Keywords: Microencapsulation, Lactobacillus plantarum, Simulated gastrointestinal conditions, Survivability, Alginate, Resistant starch

P222: **Application of Emulsion Technique for Microencapsulation of Lactobacillus plantarum in Alginate/Resistant Starch Beads**

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Background and Aim: Microencapsulation technology is commonly used to improve the viability of probiotic bacteria in foods and in the gastrointestinal adverse conditions. One of the most successful methods has been used for this purpose is emulsion technique. Resistant starch has prebiotic functionality and can improve the viability of probiotic bacteria by providing additional protection. This research aimed to evaluate the application of emulsion technique for microencapsulation of L. plantarum using combination of alginate and resistant starch.

Methods: Mixture of sodium alginate (2% W/V) and resistant starch (2% W/V) in distilled water was prepared and sterilized at 121˚ C for 15 minutes. Then pure bacterial mass (1% V/V) was added into the mixture and microcapsules were prepared by the use of emulsion method. Bead's morphology and size was studied by the use of Scan Electronic Microscope (SEM). Light microscope was used for direct observation of live entrapped bacteria inside the beads after lugol staining. The metabolic activity of entrapped bacteria was investigated by measurement of pH and optical density (OD) of 2% inoculated MRS-broth medium, over a period of 56 h incubation. Stability of beads in acid, bile salts, pancearatin enzymes and buffer phosphate solutions were studied at 37˚ C, with or without 400 rpm mechanical tension for 120 minute every 30 minute by enumeration of released bacteria.

Results: Prepared microcapsules were generally uniform and spherical shape with the mean size of 19.87 ± 1.49 µm. Results of pour plate counting indicated that prepared capsules contain 1.7 ± 0.1 × 109 CFU g-1 viable entrapped cells and Entrapment efficiency was obtained 30.35 %. Encapsulated bacteria were metabolically active and could change the pH and OD of inoculated medium. Also, their viability was approved by the direct microscopic observations of wet mount slide. Stability of beads in bile salt solution and pancreatin enzymes without mechanical tension was respectively 60 and 90 minutes and in other conditions was 30 minutes.

Conclusion: Generally it was indicated that emulsion method could be successfully applied for microencapsulation of L. plantarum using combination of alginate and resistant starch to enhance its viability in foods and gastrointestinal tract.

Keywords: Lactobacillus plantarum, Microencapsulation, Emulsion Technique, Alginate, Resistant Starch

P223: **Thermal resistance of listeria monocytogenes with NaCl in liquid egg products.**

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Background and Aim: Listeria monocytogenes is one of the most important pathogens that can cause severe illness listeriosis in humans which leads to meningitis or meningo-encephalitis, bacteriaemia and septicaemia and is highly lethal. Most cases of infection occurs through food contaminated. Listeria spp. has been found in egg products. Eggs contain a nutrients that form a suitable substrate for the growth and multiplication of Listeria spp. The best method for controlling these pathogens in egg product (liquid whole egg, liquid egg white and liquid egg yolk) is use of heat pasteurization. In this study, effect of 10% salt on heat resistance of Listeria monocytogenes in Liquid egg products was determined. To determine the heat resistance of bacteria various procedure are used such as thermal-death-time (TDT) disks, sealed tubes, flow heat exchanger, glass TDT tubes and aluminium TDT disk.

Methods: In the present study glass TDT tubes method for heat injury at 53 °C, 55 °C and 57.5 °C were used. we prepared inoculum with concentration of approx. 5×108 CFU/ml of 24 h fresh culture of the L. monocytogenes grown on TSB media at 37°C. 1 ml of this media was inoculated in to 100 ml of each liquid egg samples. Infected samples were incubated in water bath at 53°C, 55 °C and 57.5 °C and samplied at different time and restored on TSA away surface plating method. After 24 h incubated at 37°C, count of bacterial colony on TSA were measured. D-values and Z-values of L. monocytogenes were determined for both cases including plain egg products and egg products with 10% salt.

Results: In the present study inactivation kinetics of L. monocytogenes was plotted by log-linear decline in surviving cells with time. Heat destruction in egg white was fastest and listeria in homogenized liquid whole egg had medium thermal resistance. In 57.5 °C the D-value was increased from 15.04 (R2=0.998) min for plain yolk to 89.13 (R2=0.989) min for yolk plus 10% salt. 7±2-fold increase in heat resistance with addition 10% salt was observed in the other temperatures and samples.

Conclusion: Thermal destruction occurred quickly in the egg whites. In addition to the alkaline pH of egg white (pH: 9.52±0.3), it can be due to the proliferation inhibiting and cell destroying effects of lysozyme, conalbumin and avidin. Salt protect L. monocytogenes from heat. The effect of salts on thermal inactivation of microorganisms is mainly related to reduced water activity and increased osmotic pressure of the heating process. The protective effect of salt on L. monocytogenes at alkaline pH (egg white) was less than acidic conditions of egg yolk (pH: 5.9± 0.3).

Keywords: Thermal resistance- listeria monocytogenes- liquid egg products

P224: **Antibacterial activities of κ-carrageenan films incorporated with with Zataria multiflora Boiss and Mentha pulegium essential oils in liquid and vapor phase**

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Background and Aim: In the present study, antimicrobial potential of κ-carrageenan films incorporated with Zataria multiflora Boiss (ZEO) and Mentha pulegium (MEO) essential oils in liquid and vapor phase against different bacterial strains (Escherichia coli, Pseudomonas aeruginosa, salmonella typhimurium, Bacillus cereus and Staphylococcus aureus) was investigated. The chemical composition of the essential oils was also studied.

Methods: Antimicrobial activities of films were determined by both agar disk diffusion and disc volatilization methods (no direct contact between the essential oil and the medium surface). In disk diffusion method, 6 mm diameter discs were put on the previously seeded Mueller-Hinton agar medium containing approximately 108 CFU/ml of the test bacteria. After incubation, the inhibition areas were reported as the “zone of inhibition”. Disc volatilization method was conducted the same, but the discs were laid on the inside surface of the upper lid, with no direct contact between it and the bacteria strains. The chemical composition of the essential oils was studied by gas chromatography/mass spectrometry.

Results: The results showed that all composite films containing ZEO inhibited the growth of the five test bacteria, with exception of P. aeruginosa, which was not inhibited at the lowest essential oil concentration (1%). As expected, antimicrobial activity was stronger at the higher concentrations of ZEO. Among the bacteria examined, P. aeruginosa showed the highest resistance, while S. aureus was the most sensitive to ZEO-containing films, with an inhibition zone of 544 mm2. The minimum concentration of MEO at which films showed a clear zone of inhibition was 1% for S. aureus and B. cereus and 3% for the rest of the studied bacteria. The antimicrobial activity of ZEO and MEO has been attributed to their essential oils, which contain the thymol and carvacrol (ZEO) and pulegone (MEO). Carrageenan films with MEO were found not to be effective at reducing the microbial growth of the tested bacteria in vapor phase except for S. aureus and B. cereus. In contrast, ZEO-containing films were able to form a clear zone of inhibition on all bacteria at all concentrations higher than 1%, except P. aeruginosa. The inhibitory effects induced by essential oils vapors were weaker than those induced by direct contact. Entrapping the essential oil molecules in a film's polymer matrix may limit their diffusion and evaporation, thus reducing the oil's antimicrobial activities. Moreover, interpenetration of water molecules from agar medium into the film matrix results in swelling, thus gradually widens the meshes of polymer network. This leads in turn to more release of the essential oil component into the surroundings, thus resulting in higher antimicrobial activities.

Conclusion: The results of this work have shown that studied essential oils especially ZEO possess antimicrobial activities not only in direct contact with the bacteria but also their vapors are inhibitor of food pathogen growth. The great advantage of the use of ZEO and MEO in vapor phase could be reduction the organoleptic alteration induced by essential oils.

Keywords: Antimicrobial, κ-carrageenan film, Zataria multiflora Boiss, Mentha pulegium, Essential oil, Vapor phase

P225: **Modeling antimicrobial effect of grape pomace powders and extracts on S. aureus in soup using artificial neural network and fuzzy logic system**

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Background and Aim: In recent years ready-to-eat soups which prepared with hot water became popular in many countries. Vegetable soup could be considered as a functional food due to contents of various vegetable and other additives .However, this food might be dangerous, unless stored under proper conditions and prepared with a sufficient heating. Because, in these foods some bacteria may grow and produce toxins which are fairly resistant against extreme levels of heat, pH and NaCl. Also, some bacteria (spore forming bacteria) may become resistant against high temperatures and during storage may grow in suitable conditions

Methods: In this study, Antibacterial activity against Staphylococcus aureus grape pomace extract and powdered soup at concentrations of 2, 5 and 10% have been studied. Antibacterial effects of GPE were more effective than those of the GPP against the bacteria. In fact, the number of bacteria decreases with increasing concentration of grape juice. Grape pulp concentration of 10% at the end of 120 hours completely inhibited Staphylococcus aureus. The grape pomace extract and is susceptible to Staphylococcus aureus. The concentration of bacteria by 10% extract was inhibited in primary storage. Data to predict antibacterial effects against bacteria grape pomace extract and powder adaptive neural fuzzy inference system models (ANFIS) and artificial neural network (ANN) and multiple linear regression (MLR) were analyzed

Results: It was found that the ANFIS model performed better than the ANN and MLR methods for the prediction of S. aureus enumeration in the soup samples incorporated with GPP and GPE

Conclusion: This study showed that some plant materials and extracts could be used as an antimicrobial agent in a model food system. Moreover, the study results indicated that the ANFIS and ANN could successfully be used for the prediction of the bacteria counts in vegetable soup

Keywords: Grape pomace, Extract, Antimicrobial effects, Modeling , ANFIS, ANN

P226: **STUDY OF SARCOCYST CYSTS IN RAW, READY TO SELL HAMBURGERS IN TEHRAN**

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Background and Aim: Sarcocyst is one of the obligatory heteroxenous protozoa which there is sporogony and gametogony stages of parasite in the intestine of final host and schizogony stage of parasite in the muscles of intermediate host.

Methods: In this research 117 frozen raw hand made and industrial hamburgers which was ready to sell in Tehran, studied for detection of sarcocyst cysts contamination rate by Dab smear method. After defreezing, samples test for observation of macrocyst, then contacted to the surface of slid, fixed and stained with Giemsa staining method, and studied by light microscope. Results analyzed with SPSS.11 software

Results: There was macrocyst of parasite only in one hand made sample, and also there were microcyst of sarcocyst in 56 hamburgers.

Conclusion: Statistical analysis didn't show any significant difference between the contamination rate of hand made and industrial samples.

Keywords: Raw hamburgers , sarcocyst , Dab smear

P227: **Specificity of DNA Finger Printing by REP2-I Primer for the Evaluation of Genetic Variability of Probiotic Lactobacillus Strains Isolated from Tarhana**

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Background and Aim: Tarhana soup is one of the traditional foods that use on the west of Iran, since the last has medical and pharmaceutical applications. Studies indicate that Tarhana is a wealthy source of probiotic bacteria. Since application of conventional biochemical methods is often not sufficient for discrimination of closely related lactobacillus species, usage of accurate and reliable methods for detection and evaluation of genetic variability of lactobacillus at the strain level is very important. The objective of this study is molecular typing of probiotic bacteria isolated from Tarhana by 16S rRNA gene sequencing and Repetitive sequence based polymerase chain reaction (Rep-PCR).

Methods: 20 strains were isolated from Tarhana, were grown in MRS broth and incubated under anaerobic conditions (8% CO2) at 37 ˚C for 24h-48h. The total genomic DNA was extracted by MBST kit according to manufacturer's instructions. PCR-mediated amplification was carried out by degenerate primers. Sequencing of 16s rDNA was performed after purification of the PCR product. The Rep-PCR reaction by REP2-I marker was performed for genotyping of isolates. UPGMA clustering methods and PCA analysis were prformed based on Dice similarity.

Results: Isolates were deposited as novel stains of Lactobacillus casei, brevis, plantarum, and Entrococcus facium in GenBank. The 20 isolates produced different banding patterns; with 14 visualized PCR products in the range of 350 to 3000 bp. Clustering methods performed on molecular data by NTSYS software which were also supported by PCA ordination plot. The REP2-I marker grouped all isolates into four main clusters in dendrogram. In all analysis, isolates of Lactobacillus casei, brevis, plantarum, and Entrococcus facium formed four separate clusters.

Conclusion: According to the results, REP-PCR can be introduced as an accurate technique for determination of genetic diversity of the lactobacillus species. This is the first report on REP-PCR application as a phylogenetic analysis tool of probiotic bacteria in Iran.

Keywords: Tarhana, Lactobacillus, genetic diversity, NTSYS, REP2-I

P228: **Microbial contamination of tomatoes in some of restaurants in Tehran**

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Background and Aim: The botanical tomato calls “Lycopersicou esculentum” that is related to “Solanaceae” family. Also infected tomato with bacteria can cause some illness such as diarrhea and digestive disorders. In this study enumeration of mesophilic aerobic bacteria was conducted using plate count agar.

Methods: “In order to enumeration of mesophilic bacteria serial dilutions of samples were prepared using phosphate buffer phosphate (PBS) as diluents. Samples were collected in sterile polythene bag directly from selected restaurants and immediately brought to the laboratory for analysis. Samples were prepared according to the method of FAO (1979) with some modifications.

Results: This study was performed during six-month. A total of 154 samples from restaurant were collected and only 15 of them were contaminated.

Conclusion: Total count of microorganism in tomato can be indicated contamination of these products that can be easily prevented.

Keywords: Tomato _resturents_illness

P229: **Antibacterial efficacy of Sweet Marjoram (Origanum majorana) essential oil on Escherichia coli O157 H7 by different susceptibility testing methods**

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Background and Aim: Nowadays, negative consumer perception about possible toxicity and other side effects of chemical preservatives, shift attentions towards natural alternatives. Essential oils are natural chemical sources used for preserving food. Most Escherichia coli strains are harmless, but some serotypes can cause serious food poisoning in humans, and are occasionally responsible for product recalls due to food contamination.

Methods: In this study, the Sweet Marjoram (Origanum majorana) essential oil was achieved by hydrodistillation method and the antibacterial effect of the essential oil on Escherichia coli O157 H7 was evaluated by disk diffusion, macrodilution and microdilution methods

Results: According to the results obtained, in disk diffusion method 34 mm inhibition zone was observed and MIC (minimum inhibitory concentrations) of the essential oil was evaluated 350 ppm in both micro and macrodilution method. Also, MBC (minimum bactericidal concentrations) was calculated 300 ppm for microdilution method and 350 for macrodilution method.

Conclusion: Based on our findings, this essential oil has a high antibacterial effect on E. coli and can be applied as a preservative in food. However, more examinations are required for practical use of this plant EO in commercial scale.

Keywords: Sweet Marjoram essential oil, antibacterial effect, Escherichia coli, susceptibility test.

P230: **Importance of salmonella in food stuff**

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Background and Aim:: salmonella is the main cause of different clinical syndromes from gastroenteritis to typhoid . The most common transmission way to human is by food stuff specially animal products . The aim of this revisal study is recognition the most important salmonella contamination sources in Iran, from 78 articles related to salmonella which are shown in www.iranmedex .ir There are 16 articles related to frequency of salmonella in food stuff . Contamination in shell surfaces eggs were 5-7 in thousand and isolated only one case from 500 considered egg. Other studied food stuff include ice cream , water, traditional cheese prepared food no contamination was reported . In studies ,The most common reported salmonella species were S.paratyphy C ,S.enteritids ,S.typhimurium .

Methods: The most common transmission way to human is by food stuff specially animal products . The aim of this revisal study is recognition the most important salmonella contamination sources in Iran, from 78 articles related to salmonella which are shown in www.iranmedex .ir There are 16 articles related to frequency of salmonella in food stuff .

Results:: salmonella contamination of poultry carcass in different studies was variable from 8/6% to %57/5 .And this scale contamination in red meet was variable from %0/75 until %2.

Conclusion: The sources indicates that in Iran there are the most risk of salmonella contamination is in hens proper process of hens paying attention to transmission cycle and sufficient cook of this products can decrease prevalence of salmonella diseases in society.

Keywords: salmonella – food stuff - hen

P231: **Production of natural lactons as a bioflavor by yeasts**

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Background and Aim: Abstract: Flavors play an important role in food industries. In dairy industry, flavor is a unique attribute which determines its acceptability. Also flavor can be described as the combined perception of taste, smell and mouth feel, Moreover flavors increase consumers response towards “Natural” ingredients. However, chemical synthesis results many problems, such as environmentally unfriendly production processes and undesirable racemic mixture compounds. Hence, biotechnology of flavors shows their necessity. Objective: The aim of this study was is to research the abilities of some yeasts such as Saccharomyces cerevisiae and Yarrowia lipolitica to produce deca-lactone and decanolide as a stabilized natural flavor for using in dairy products.

Methods: Material and methods: For production of d-decalactone, The α,β-unsaturated lactone which is the main component of Massoi bark oil, can efficiently converted by Saccharomyces cerevisiae into d-decalactone in a controlled biological process. But Most of the commercial processes for the production of decanolide are based on the Ricinoleic Acids)Natural Hydroxyl Fatty Acids(. Ricinoleic acid, the main fatty acid of castor oil, or esters thereof as substrates and fatty acid degrading yeasts (e.g. Yarrowia lipolitica) or a higher fungi as biocatalysts.

Results: Results: The process for which high product concentrations have been reported by Haarmen and Reimer are based on strains of Yarrowia lipolytica, a yeast which is particularly well adapted to hydrophobic environments and which was patented for 4-decanolide production for the first time. Ricinoleic acid, is degraded by four cycles of ?-oxidation and one double-bond hydrogenation into 4-hydroxydecanoic acid, which lactonises at lower pH to 4-decanolide, resulting in the same R configuration of the lactone as is found in peaches and other fruits . By controlling the process, they could obtain up to 11 g/L 4-decanolide in 55 h with raw castor oil as the substrate by Yarrowia lipolytica. Subsequently producing of this bioflavor has resulted US $ 300 per kilogram. Also octanolide naturally found in meat, cheese, can be produced as a by-product besides decanolide when a mixture of 11-hydroxypalmitic acid and 3,11-dihydroxymyristic acid from Jalap resin is converted by Saccharomyces cerevisiae.

Conclusion: Conclusion: Biotechnological processes can be used as an alternative to natural compounds, it is essential to know that the use of bioflavors can enhance the safety and quality of foods. Hence work is needed for future developments.

Keywords: Bioflavor, Yeasts, Natural lactons, Yarrowia lipolytica, Saccharomyces cerevisiae

P232: **The Effect of fermentation of celery juice and apple juice by probiotic on organic acids and sensory properties**

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Background and Aim: Abstract: Recently the production of probiotic beverages from selected bacterial strains, such as Lactic acid bacteria, is a new controlled fermentation process. In this research the ability of Lactobacillus acidophilus and Lactobacillus delbruekii on celery juice and apple juice for producing organic acids and their effect on sensory properties (color, taste, texture, odor and density) were evaluated.

Methods: Materials and methods: Two strains of LAB in this study were cultured in MRS Broth and MRS Agar at 37 c° for 24 h. Chemical changes were measured by HPLC and sensory properties were determined by Hedonic 5 points and by preparing a formulation in 3 proportions (15, 30, 45%) of fermented juices with 5 professional assessors. All experiments were carried in Karaj branch of University of Tehran.

Results: Results: Results showed that during the fermentation course of all juices, the highest increase in cell number was 9.001 Log (cfu/ml) in celery juice by L. acidophilus and the lowest was for apple juice by L. delbruekii (7.525 Log cfu/ml). Organic acids production (Lactic, Acetic and Gluconic) in celery juice by L. acidophilus (11.14, 4.00, 3.22 g/L) in comparison to the apple juice observed the highest amount. Also, the 15% proportion in fermented celery juice by L. Acidophilus, acquired the highest score by the analysts for color, taste and odor properties. In the 30% fermented apple juice proportion, color and odor properties acquired the best score. The final 45% proportion in both fermented juices, was considered undesirable by the analysts.

Conclusion: Conclusion: As far as LAB obtain energy only from the metabolism of sugars, lactic acid bacteria are restricted to environments in which sugars are present. They have limited biosynthetic ability, having evolved in environments that are rich in complex medium that fulfill all their nutritional requirements and specially because celery juice's medium as a substrate was poor in sugary substances, but we noticed that Both species of LAB, L. acidophilus and L. delbruekii were capable of growing well and producing organic acids with acceptable sensory properties in celery and apple juices. L. acidophilus in celery juice and L. delbruekii in apple juice had the highest lactic acid productions. Also the 15% proportions in both fermented juices, acquired the highest score for producing a non-dairy fermented beverage.

Keywords: fermented juices,celery juice,apple juice,probiotic,Lactic acid bacteria

P233: **Microbial biosurfactants and their importance & applications in food industry**

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Background and Aim: Abstract: Biosurfactants or microbial surfactants are surface active biomolecules that are produced by a variety of microorganisms like bacteria and fungi . These molecules are amphiphilic compounds which reduce surface and interfacial tensions in both aqueous solutions and hydrocarbon mixtures and mainly classified according to their molecular weight, physico-chemical properties and mode of action. Rhamnolipids from Pseudomonas aeruginosa, Trehalolipids from Mycobacterium, Nocardia, and Corynebacterium, Sophorolipids mainly from yeasts such as Torulopsis bombicola T. petrophilum and T. apicola are some examples of microbial-derived surfactants. Objective: In this paper, we reviewed the current knowledge and the latest advances in biosurfactant applications and importance in food industry.

Methods: Material and methods: According to the researches, these microbial compounds(BS) exhibit a variety of useful properties for the food industry specially as emulsifiers, foaming, wetting, solubilizers antiadhesive and antimicrobial agents.For example the lipopeptide iturin from B. subtilis showed potent antifungal activity or Rhamnolipids from Pseudomonas aeruginosa and Sophorolipids mainly from yeasts such as Torulopsis bombicola T. petrophilum and T. apicola were found to be effective antifungal agents. Due to controlling the adherence of microorganisms to food contact surfaces is an essential step in providing safe and quality products to consumers, The bioconditioning of surfaces through the use of microbial surfactants have been suggested as a new strategy to reduce adhesion and the involvement of biosurfactants in microbial adhesion and detachment from surfaces has been investigated.

Results: Results: Interest in biosurfactants has increased considerably in recent years, as they are potential candidates for many commercial applications in food processing industries owing to their unique properties and special advantages over synthetic surfactants – such as lower toxicity; higher biodegradability; better environmental compatibility; higher foaming; high selectivity and specific activity at extreme temperatures, pH and salinity; also the ability to be synthesized from renewable feedstocks as they present a much broader range of surfactant types .In addition Some of these biosurfactants can be used as food formulation ingredients due to their obvious role as agents that decrease surface and interfacial tension, thus promoting the formation and stabilization of emulsions and surfactants. biosurfactants in bakery and ice cream formulations, act controlling consistency, retarding staling and solubilizing flavor oils. Considering to our research these biosurfactants can be play an efficient role in food industries.

Conclusion: Conclusion: Biosurfactants show several properties which could be useful in many fields of food industry and could be explored in food processing and formulation. Potential applications of microbial surfactants in food area and the use of agroindustrial wastes as alternative substrates for their production can be discussed in future studies.

Keywords: Keywords: biosurfactants, Surfactants ,microorganism, applications, food industry

P234: **Antibacterial activity of some essential oil plants on food borne disease bacteria**

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Background and Aim: Investigation on compounds and materials with antimacrobial activity performs continuously. Drug plants have become important in medical field and food industry as a food preservative. In this review article we explain some studies about antibacterial effect of essential oil of cinamon,oregano,rosmary,clove and thyme.

Methods: Antibacterial activity of plant essential oils of these plants were tested by Agar well diffusion method, Minimum inhibitory concentration,MIC,and Minimum bactericidal concentration,MBC,

Results: According to the results of scientists, essential oils of cinamon,clove,oregano,rosmary and thyme had inhibitory effect against food borne bacterial such as campylobacter sp, listeria monocytogenes,Yersinia, Salmonella sp. Among all essential oils, clove oil exhibited the strongest antibacterial activity followed by cinamon,thyme, organo and rosmary.

Conclusion: The essential oil of clove, cinamon and thyme is a potential source of natural antibacterial agents and to be used as food preservative.

Keywords: Antibacterial- essential oil- food

P235: **The survey of reasons and microbial contamination milks pasteurized in milk factories in Markazi province**

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Background and Aim: Milk had a major role in human nutrition is so important to control and monitor the health standards. Coliform and E. coli are important for milk quality and their presence is considered as an indicator of fecal contamination

Methods: This cross-sectional study was conducted. Cluster sampling, 300 samples of milk from 10 units of the factory in the Markazy province during a year .the sample and the presence of E. coli, total count of microorganisms and coliform were analyzed according to the national standard. Data were analyzed with SPSS statistical software

Results: The results showed that of 300 samples of milk tested for coliform count and the total count of microorganisms in 56 (18.7%) and 48 (16%) was higher than the permissible limit.42(14%) are contaminated to Escherichia coli.General microbial contamination of milk with 78 samples (26%) were identified. Also the results showed that contamination of pasteurized milk was significantly higher in summer ($p<0.005$). Acoording to cheque list of the production facilities evaluation there was a significant correlation($p<0.005$) between microbial contamination of pasteurized milk with points lower factory in maintenance program for the machine production line especially with pasteurizer, disinfection and cleaning of equipments production line, program for disinfection and cleaning of equipments production line, having health care workers, having sanitary toilets.

Conclusion: According to the research is necessary having program to repair the machines in the production line ,high efficiency pasteurizer , compliance washing, cleaning and disinfection of equipment used in the production line, Having an efficient program for washing, cleaning and disinfection of equipment used in the production line, Workers use of hats, special clothing and shoes, mask and Timely cleaning workers. therefore, in order to produce good quality pasteurized milk is essential coordination with relevant organizations to resolve nonconforming items.

Keywords:: Milk pasteurized , Escherichia coli, Coliform, Total count of microorganisms,Markazi province

P236: **Evaluation of microbial contamination of pastry cream in Arak, Iran**

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Background and Aim: cream and pastry cream are used very much and also are important part of production guild. Contaminations of whipped cream are even more then pastry cream. Microbial Contaminations causes human to have contagious diseases by the digestive system. Of converse mentioned diseases depend on methods of making and keeping of cream. Food habits, health care by persons who making pastry cream and primary and secondly Contamination of cream. The goal of this study is consideration of pastry cream microbial Contamination at confectioneries

Methods: In this study, total of 120 samples were randomly obtained from confectioneries and analyzed for microbial contamination levels, according to Iran's national standards

Results: In this research discovered that among 120 samples which were tested, 115 sampled (%95.8) are non – use and contaminated to various microbes . That 98 samples are (%81.6) contaminated to entrobacteriacea (>1×102 CFU/Gr), 5 sample (%4.2) are contaminated to mold (>3×102 CFU/Gr), 115 samples (%95.8) are contaminated to yeast (>1×103 CFU/Gr). 36 samples (%30) are contaminated to Escherichia coli (negative), there is no salmonella in pastry creams (negative).

Conclusion: in fact, this study clears those 115 samples of whole samples of Arak's pastry creams are contaminated to various microbes and are non – use . This fact shows non – health conditions of production workshops and no health observe at working and keeping the produce. The most contaminations related to Yeast and Entrobacteriaces.

Keywords: Arak, Confectionery, Microbial contamination, Pastry creams

P237: **Use of date waste and soybean meal for single cell oil production by Mortierella alpina in solid substrate**

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Background and Aim: Single cell oil (SCO) production by species of Mortierella, especially M. alpina, has been of great importance for some agricultural and food industry wastes using solid state fermentation (SSF). SCO contains a wide range of polyunsaturated fatty acids (PUFAs) which are considered to have a significant effect on human health. Date waste and soybean meal are rich in nutrients and could be used as carbon and nitrogen sources for SCO production.

Methods: In this research, SCO production was performed by M. alpina CBS 528.72 in appropriate conditions after the analysis of substrate components, including content of moisture, pH, ash, some minerals, fatty acids and oil. Date waste and soybean meal were used as sources of carbon and nitrogen in SSF, respectively.

Results: The results showed that ratio of organic carbon to total nitrogen of the date waste was not appropriate for SCO production by this fungus and additional nitrogen source was required. Therefore, in the presence of soybean meal as a nitrogen source, PUFAs-rich SCO was produced based on dry fermented substrate 6.54 %.

Conclusion: It is evident that conversion of wastes of agricultural and food industry into valuable materials is a very important step to solving problems of wastes produced in the country.

Keywords: Solid substrate, Date waste, Single cell oil, Soybean meal, Mortierella alpina.

P238: **Determination of effective process factors on pulyunsaturated fatty acids production by Mortierella alpina in solid state fermentation using date waste**

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Background and Aim: Polyunsaturated fatty acids (PUFAs) are important components of cellular structure and function and serve as precursors to eicosanoids, including prostaglandins and leukotrienes. The fungus Mortierelh alpina is able to produce these biologically active components. Culture media and culture conditions affect quality and quantity of PUFAs production. Results of previous researchers have indicated that solid state fermentation (SSF) could be useful in fungal lipid production. With SSF, it is possible to utilize renewable and low cost natural resources, such as agricultural and wood remains, energy crops and by-products of food industry.

Methods: PUFAs production was performed on date waste by Mortierell alpina CBS 528.72 in solid state fermentation (SSF) using Plackett Burman Design (PBD). Effect of eleven variables including substrate particle size, initial moisture content and pH of substrate, carbon to nitrogen ratio of substrate, supplementations of soybean oil (SBO) and linseed oil (LSO), inoculation age, incubation temperature and time, heating pre-treatment time and supplementation of the nitrogen on the fourth day were also investigated.

Results: In the range of studied variables in this research, addition of 10 % (w.w-1) LSO and 10 % (w.w-1) SBO, incubation time of 12 days and initial pH of substrate 6 had a significant effect on the increasing production of PUFAs. Maximum of total PUFAs produced in this study was 65.69 % in dry fermented substrate including 15.98 % linoleic acid (LA), 1.80 % gamma linolenic acid (GLA), 29.34 % alpha linolenic acid (ALA), 12.77 % arachidonic acid (ARA), 5.09 % eicosapentaenoic acid (EPA) and 0.40 % docosahexaenoic acid (DHA).

Conclusion: Therefore, production of the maximum amount of PUFAs by M. alpina can be achieved to modify the date waste substrate considering the above-mentioned factors.

Keywords: Poly unsaturated fatty acids (PUFAs), Mortierella alpina, Solid state fermentation (SSF), Date waste.

P239: **Estimation of Microbial Quality of Vegetable salads**

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Background and Aim: Consumption of vegetable salads has increased in worldwide. Because of high nutritional value in vegetables, they are an important part of diet. Unfortunately, the increase of consumption of vegetable salads has come with an increased frequency of outbreaks of food born disease. The aim of this study was to evaluate the bacteriological quality of vegetable salads.

Methods: This study was performed on 222 samples vegetable salads sent to the laboratory of control drug and food Tehran University of medical science. The samples were analyzed for mesophilic aerobic bacterial count, enterococcus count and mould count, as well as for the presence of Escherichia coli, Staphylococcus aureous and Salmonella, according to the standard guideline.

Results: 30% (65/222) of the samples were not acceptable microbial quality. The highest contamination rate was related to the total count of bacteria 70% (46/65) and , enterococcus count 45% (31/65) and the lowest prevalence of contamination due to Mould count 9%(6/65). In contaminated samples, 23% (15/65) were infected with E. coli and 2% (1/65) with S.aureus but no salmonella contamination was observed in tested samples.

Conclusion: Sources of microbial contamination in this study could be due to the non-compliance with disinfection and or because of secondary contamination in washed vegetables.Training of individuals involved in the preparation of salads is essential for reducing contamination. Bacterial pathogens such as E. coli contamination of salads can be induced by manipulating people while preparing the salads. However, these salads can be stored for long periods in bad temperature and opportunistic pathogens grow, and in such cases, lack of sanitation can cause outbreaks of food poisoning.

Keywords: Microbial Quality, Vegetable salads, Disinfection

P240: **Investigation of enteric Gram-negative bacilli contamination in Mazafati Date**

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Background and Aim: Food safety is related to the existence and level of hazards of foods used. World Health Organization (WHO) considers diseases caused by the consumption of contaminated food as the most important and serious problem of today's world. Therefore, appropriate sanitary measures are necessary to avoid human health risks. In addition to domestic consumption, dates are one of the major and significant Iranian agricultural exported products in Iran's non-oil category

Methods: Hundred 500-gram packages of date were randomly sampled and tested from the date distribution centers. After appropriate dilution, bacteriological tests were performed. Coliform count of the solid medium (VRB), Enterobacteriaceae count of solid medium (VRBD), E.coli from liquid medium LS+ as well as confirmatory tests to detect of enterococcus from bromocresol liguid medium-purple azide broth culture as well as KF solid medium for confirmatory tests were used

Results: After reviewing the results, only 11% of the samples had coliform contamination. There were no E.coli, Enterobacteriaceae and enterococcus contamination in the samples

Conclusion: Our finding, thus, confirms the antibacterial effects of date; however it may require further investigation in this area.

Keywords: Date, coliform, E.coli, Enterobacteriaceae, enterococcus

P241: **Studies of Antimicrobial Activity of Turmeric and Cinnamon**

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Background and Aim: Plant oils and other extractions have been used throughout the evolution by humans for reasons such as food preservation, medicine, and etc. Since then plant extracts have come a long way and while they satisfy their original purpose, they have also gifted human kind with far more advanced benefits as well. Cinnamon plant is a native plant of the tropical islands and tumeric is native to tropical south Asia. Both of them considered as herbs and spices traditionally used by many ancient cultures.

Methods: Antibacterial activity of these extracts was determined against several different bacteria using Well Diffusion Agar (WDA) method. The powders were weighed and extracted with methanol and distilled water separately. They were soaked in respective solvents for 3 days and then filtered with Whatman filter paper (pore size 0.2µm). same amounts of extracts were used for further studies such as antibacterial activity.

Results: Various studies revealed different degrees of antimicrobial activity, with essential oils and aqueous extracts of Turmeric and Cinnamon showing the greatest potential. Antimicrobial activity was tested against E.coli,Salmonella,Pseudomonas,Staphyloccus,Klebciella,Bacillus,Shigella. Between methanol and Aqueous extracts of Turmeric and Cin namon, Aqueous extracts showed good antimicrobial effect against pathogen bacteria.

Conclusion: In particular, the antimicrobial activity of plant extracts has formed the basis of many applications, including raw and processed food preservation, pharmaceuticals, and alternative medicine. In fact, plants produce a diverse range of bioactive molecules,making them a rich source of different types of medicines.Plants with possible antimicrobial activity should be tested against an appropriate microbial model to confirm the activity and to ascertain the parameters associated with it.

Keywords: Antibacterial,cinnamon,turmeric

P242: **Occurrence of Salmonella spp. in Qazvin Table eggs**

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Background and Aim: Salmonella is a major food-borne bacterial pathogen that has most often been associated with consumption of contaminated foods of animal origin, such as poultry, dairy products and eggs. Poultry are considered an important source of Salmonella food-borne disease and the illnesses were associated with the consumption of contaminated eggs. Infected ovaries and oviducts of the hen are the major sources of contamination. The aim of this study was to compare the prevalence rate of salmonella on egg content by using conventional microbiology detection.

Methods: 350 Egg samples of 21 poultry farms were selected and were examined between March 2012 and March 2013. Salmonella organisms were isolated according to standard methods (ISIRI 1810, 2002). Samples of 25 g whole egg were placed in a stomacher bag, containing 225 ml of pre-enrichment buffered peptone water, treated in a Stomacher for 2 min and incubated for 16-20 h at 37°C. 1 ml of pre-enriched cultures, were transferred to Rappaport- vassiliadis broth and also to Tetrathionat -novobiocine broth and incubated at 42 °C. After 24 h of incubation, one loopful from each of enriched broths was streaked onto plates of Salmonella-Shigella (SS) agar and xylose-lysine deoxycholate (XLD) agar and incubated at 37 °C for 24 h. The plates were examined for the presence of typical colonies of Salmonella. Transparent colonies with black center on SS-agar and red colonies with black center on XLD agar. Suspected colonies were confirmed by conventional biochemical methods.

Results: The results showed that two of the samples (0.0057%) were contaminated with Salmonella organism.

Conclusion: According to the results obtained in this study and in order to prevent outbreaks of Salmonella infections in humans caused by consumption of eggs, Management practices should be applied to prevent or reduce the spread of salmonella infections in farms.

Keywords: salmonella , egg , culture , contaminated

P243: **Inhibitory Effect of Garlic Extract on the Growth of Salmonella Typhimurium and Shigella Dysenteric**

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Background and Aim: Nowadays, using plant extracts as antimicrobial additives has got an important role in maintaining the quality of food products. Garlic is one of the plants the antimicrobial effect of which has been proved by biochemical investigation. The aim of this study was to assess the effect of different concentrations of garlic powder extract and garlic tablet extract on Salmonella typhimurium and Shigella dysenteric in the same conditions.

Methods: To do this investigation, fresh garlic from Hamadan and garlic tablets from Kowsar Pharmacy Company was provided. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of garlic Powder extract and garlic tablet extract on the growth of two micro-organisms were tested through tube standard method.

Results: The MIC of garlic Powder extract on the tested microorganisms was 12.5 mg/ml and the MBC was 25 mg/ml, while the MIC of garlic tablet extract was 40mg/ml and the MBC was 80 mg/ml. According to these results, the inhibitory effect of the extract of the garlic (GP >) on both bacteria was similar, but the inhibitory effect of the garlic powder was much more than that of garlic tablet (3.28 times), either on Salmonella or Shigella.

Conclusion: Noticing the findings of the present study and other related reports in this field, the application of this extract in food preservative systems is useful for inhibiting food contaminations.

Keywords: Garlic extract, Salmonella typhimurium, Shigella dysanteria, Minimum inhibitory concentration,Minimum bactericidal concentration.

P244: **A study on microbial contamination of ready to eat foods in Isfahan city by conventional and molecular methods**

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Background and Aim: Since most of ready to eat foods such as nuts, confectioneries and fruit juice are sold in bulk or without appropriate packaging, they have high potential for containing microbial pathogens which might cause health problems for consumers. The aim of this study was to determine the bacterial and fungal quality of ready to eat foods distributed in Isfahan local shops.

Methods: In the current study, 100 samples, including 78 snack nuts and confectionery and 12 juice samples were taken randomly from food shops in Isfahan. The samples were assessed for microbial contamination by Aerobic Plate Count (APC) in search of major pathogens, i.e., Enterobacteriacea, Staphylococcus aureus, Salmonella, Shigella, Escherichia coli and fungal organisms. The 16S rDNA and 18S rDNA were used for identification of unknown species of bacterial and fungal organisms by conventional methods.

Results: Our data showed that 26.13% of snack nuts and confectioneries and 91.67% of juice samples were contaminated with enteric pathogens. We also observed that 18.18% of snack nut and confectioneries and 8.33% of juice samples were contaminated with Staphylococcus aureus. We found no Salmonella and Shigella contamination in ready to eat food samples. The Fruit juice was found to be the most contaminated foods with respect to fungal contamination (75%). the snack nuts and confectioneries ranked the second (56.81%).

Conclusion: The findings of the present study, verify that the ready to eat foods are at risk of high contamination which might pose substantial health risks for Iranian consumers. We recommend that proper food packaging and training of food handlers are essential to avoid microbial contamination of ready to enact foods.

Keywords: Food Contamination, Ready to eat foods, Aerobic Plate Count

P245: **A study on the probiotic activity of Enterococcus hirae isolated from Iranian dairy products**

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Background and Aim: Lactic acid bacteria have traditionally been used in food processing because of their potential activity to improve the organoleptic characteristics and healthiness of foodstuffs. It has been demonstrated that different antimicrobial agents produced by these bacteria can inhibit pathogenic and spoilage microorganisms, extending the shelf-life and enhancing the safety of food products. Hence, the current research was conducted in order to isolate lactic acid bacteria producing antibacterial agents from Iranian dairy products.

Methods: Dairy samples were aseptically collected from rural households of different central region of Iran. For antimicrobial activity detection, agar spot test was performed. The cell free supernatants of isolated strains suspected of bacteriocin producing were adjusted to proteolytic enzyme treatments. The isolated bacteria were identified by Gram-staining and biochemical tests. The MIC of antibacterial agent was determined by agar well diffusion assay.

Results: The isolated bacteria was identified as Enterococcus hirae which showed inhibitory activity towards E. coli and S. aureus as Gram negative and Gram positive indicators, respectively. The inhibitory activity was not affected by catalase treatment and was preserved in neutralized supernatant fluid. This suggests that hydrogen peroxide did not involve in the inhibition and apart from organic acid, the activity could be related to the production of bacteriocin-like inhibitory substances. Treatment of samples with proteinase K and pepsin induced complete inactivation of the antimicrobial activity indicating proteinaceous nature of the secreted compound.

Conclusion: Recognition of bacteriocins from Iranian traditional dairy products could lead us to apply new antimicrobial substances for controlling the microbial quality of food products.

Keywords: Lactic acid bacteria, Bacteriocin, Minimum inhibitory concentration, Dairy products

P246: **Study on microbial contamination of packaged natural mineral and drinking waters**

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Background and Aim: According to food and drug administration (FDA), packaged water has been defined as water that is sealed in bottles or other containers and intended for human consumption. There are two different categories of packaged water, natural mineral water and all other kinds of bottled water. The source, process and also microbiological criteria of these two kinds are different.In general the requirements requested for natural mineral water are more strict than those required for drinking water due to the source and specific process. These specification in drinking water are Coliforms and Escherichia coli , but in natural mineral water, in addition to these bacteria, Pseudomonas aueroginosa. Faecal Enterococcus and sulfate reducing bacteria are included.

Methods: According to National standards of Iran

Results: In this article the status of microbial contamination of packaged drinking water (natural mineral water and drinking water) from different regions of country were studied

Conclusion: The result of survey expressed that most of the contaminated cases were natural mineral water that contaminated samples had pseudomonas aueroginosa

Keywords: contamination,mineral water, drinking water

P247: **Effect of different concentration of Ziziphus honey on growth of Escherichia coli and Staphylococcus aureus**

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Background and Aim: Honey exhibits antibacterial properties. The growth of bacteria is inhibited due to the low water activity, high acidity, and the hydrogen peroxide activity in honey. Also honey contains natural antioxidant properties. E. coli and S. aureus are from the most common causes of enteritis and food poisoning worldwide. In attention to these properties in honey, the present investigation was performed to determine the antibacterial activity of Ziziphus honey against E. coli and S. aureus.

Methods: In present research, a natural Ziziphus honey in 10%, 20%, 30% and 40% dilutions (v/v) provided in nutrient broth medium. Then about 106 CFU/ml of E. coli and S. aureus separately added to experimental trials and incubated at 35 degree centigrade for 120 hours. Viable count enumeration of the sample was investigated after 0, 24, 72 and 120 hours post inoculation.

Results: The results showed that antibacterial activity of Ziziphus honey against S. aureus was more than E. coli. In a comparative trial, antibacterial activity of Ziziphus honey was higher after 120 hours incubation for each two bacteria in all dilutions.

Conclusion: The statistical analysis by SPSS 16 showed no any significant difference between 24, 72 and 120 hours incubation on S. aureus in 40% dilution. For the both bacteria, antibacterial activity was increased with addition concentration of honey. The microbial count showed 7.5 and 7 log reduction in S. aureus and E. coli population respectively comparing with control after 120 hours. Therefore it is recommended using Ziziphus honey as a natural preservative, antioxidant and antibacterial agent.

Keywords: Ziziphus honey, Antibacterial activity, Escherichia coli, Staphylococcus aureus

P248: **Isolation and identification of salmonella from Tigertooth croaker fish (Otolithes rubber) in Ahvaz market**

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Background and Aim: Salmonellosis is one of the most important zoonoses diseases. Salmonella species are major enteric pathogens that cause of gastroenteritis and fatal septicemia in human. Salmonella species are known as food borne bacteria because infected of human by foods. Fish and shrimp and other aquatic organisms are very good Resources for these kind of bacteria. These bacteria have been isolated from fresh and processing aquatic foods in the most countries.

Methods: In this research 60 Tigertooth croaker fish in Ahvaz market were collected and carried to laboratory. Then two different organs include intestines and gills from each fish at sterile condition prepared. For detecting Salmonella after culturing in Lactose Broth, Selenit F and Tetrathionat according to the reference conventional methods, the suspected colonies were inoculated in TSI, Urea and LD agar. Then the serological tests were carried out on suspected colonies.

Results: The results showed that 2 (3/3%) samples were contaminated to salmonella. S. enteritidis (B and C serogroups) were detected in the serological tests.

Conclusion: These pathogenic bacteria have been contaminated Khouzestan coastal zone through waste water, Chemical industrial and human sources. Therefore people receive these kind bacteria through consumption of aquatic contaminated foods. In attention to the public health importance of salmonella, more attention should be considered to eliminate the waters contamination by controlling entrance ways.

Keywords: Salmonella, Tigertooth croaker fish, Ahvaz market

P249: **Viability of Lactobacillus casei in Yoghurt Containing different hydrocolloids**

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Background and Aim: Probiotics are Live microorganisms which when administered in adequate amounts confer a health benefit on the host. probiotic cultures are available in fermented products and probiotic fortified foods. Lactic acid bacteria (LAB) and bifidobacteria are the most common types of microbes used as probiotics. Among the different foods act as a probiotic carrier, dairy products have special importance. Yoghurt is the most popular fermented milk product in the most parts of the world and commercially produced by inoculation of milk with a mixture of Lactobacillus bulgaricus and Streptococcus thermophilus cultures.

Methods: In the current study viability of Lactobacillus casei as a probiotic culture have been surveyed in the yoghurts treated with gelatin, corn starch and inulin in the levels of 0.4 and 0.8% (w/w) at 0, 7, 14 and 21 days of storage intervals. In addition, texture, organoleptic and chemical characteristics of probiotic and commercial yoghurts have been comprised.Viable cells were enumerated by plating diluted samples (peptonized water) on solid MRS agar. Cultures were incubated for 72 h at 37 °C to determine Lactobacillus casei population.

Results: The results showed that the numbers of Lactobacillus casei in the yoghurt samples were 8.59 log cfu/gr at the first day of production. These amounts reached to 5.55 log cfu/gr in control sample (without hydrocolloid) and 6.11, 6.19 and 6.05 log cfu/gr in yoghurts contain inulin, gelatin, corn starch after 21 days of storage time respectively. At the end of 21th day, the number of live probiotic cells were higher than those recommended for beneficial effect in the treated samples.

Conclusion: Viability of probiotic bacteria were significantly ($P<0.05$) higher in yoghurts containing 0.8% hydrocolloids. The result of texture analysis which conducted with a rotational viscometer(Brookfield viscometer) and sensory evaluation showed that yoghurts treated with gelatin had higher viscosity and acceptable flavor in comparison with others. Therefore gelatin can be used successfully in the production of probiotic yoghurt with Lactobacillus casei without any adverse effect on quality during storage.

Keywords: Viability, Lactobacillus casei, Yoghurt, Hydrocolloids

P250: **Genotypic Characterization of lactic acid bacteria Isolated from traditional cheese in Khorramabad city of Iran.**

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Background and Aim: starter cultures are required for the industrial production of cheese. These starter cultures are mainly composed of lactic acid bacteria. They produce lactic acid during the fermentation process and produce the curd from milk Traditionally, milk from cow, goat and sheep is fermented by the help of naturally occurring indigenous lactic acid bacteria.The aim of this project was to isolate and identify natural lactic acid bacteria flora involved in traditional fermentation in cheeses of Khoramabad city with genotypic method.

Methods: Isolated with negative catalase and positive gram isolation and Genomic DNA from pure cultures was extracted with the bioneer kit (korea), The PCR amplification of approximately 2000 bp of the ITS region of the 16S rRNA gene was obtained. At the end The PCR product for sequencing was sent to bioneer co in seoul, south korea.

Results: At the end of study, PCR-Sequencing of the 16S rRNA genes of the 4 isolates and search for homology in the NCBI-BLAST database showed that Isolate DC67 and KC100 showed 99% similarity with the 16S rRNA genes of Enterococcus. faecium DO chromosome, Isolate GC93 showed 99% similarity with the 16S rRNA genes of Enterococcus. hirae ATCC 9790 chromosome, Isolate EC55 showed 96% similarity with the 16S rRNA genes of Lactococcus lactis subsp. cremoris UC509.9 chromosome, Isolate GB2 showed 99% similarity with the 16S rRNA genes of Lactobacillus plantarum subsp. Plantarum ST-III.

Conclusion: This study showed that there are of lactic acid bacteria in the traditional cheese of the Khorramabad city that can using as starter bacteria in fermentation production.

Keywords: lactic acid bacteria, probiotic, PCR, Sequencing

P251: **Encapsulation of live bacteria and viruses by electrospinning or electrospraying**

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Background and Aim: The encapsulation of biological material in a dry form while preserving its activity is important for many applications. The most common encapsulation techniques are spray-drying, emulsifying-crosslinking or coacervation. New technologies which do not involve severe conditions, in terms of temperature and solvents used, and that give rise to smaller capsule sizes are highly desired. In this sense, high-voltage spinning commonly known as electrospinning is a simple and highly versatile method to produce fibres and/or capsules in the micro, sub-micro and nano range (in the case of the production of capsules the technique is also known as electrospraying), presenting a large surface to volume ratio. Electrospinning is a method to prepare long, thin nanofibres, with diameters ranging from tens to hundreds of nanometers, from polymeric solutions. This technique, has a tremendous potential in the food science area for development of novel functional ingredients. The spinning solution may contain particles, emulsion droplets or microorganisms, which after spinning end up in an encapsulated state in the fibres.

Methods: The electrospinning process involves the application of an electrical field by high voltage to continuously draw droplets of viscous polymer solution from a syringe needle towards a grounded collector. In the presence of an electrical field applied to the solution, the Maxwell electrical stress stretches the droplet, a Taylor cone is made and jetting sets in. The solvent eventually evaporates, the jet dries and solidifies, and the nanofibres are deposited on the counter electrode. Processing parameters such as applied voltage, solution feed rate and the distance between needle tip and collector are believed to have an effect on the morphology of the electrospun fibers. Regarding the encapsulation matrices, proteins and polysaccharides have attracted a considerable interest because these natural biopolymers are surface active materials. Moreover, they may be considered as amphiphilic macromolecules that play an essential role in stabilizing food formulations.

Results: Several studies used the electrospinning technique to embed both spherical and rod-likebacteria and bacterial viruses (e.g. probiotics like bidobacterium, bacteriophage T4, yoghurt cultures) in a polymer matrix, which forms a composite nanofibre during electrospinning. The bacteria or viruses are initially dispersed in a polymer solution and randomly oriented. Due to the sink-like flow at the Taylor cone, the rod-like bacteria and viruses are gradually oriented, mainly along the stream lines, so that aligned organisms are pulled into the jet in an almost oriented manner.

Conclusion: Hydrocolloid-based sub-micro and microcapsules have been developed through an electrospinning/electrospraying process for the protection of living microorganisms. Therefore, this technique may represent an excellent alternative to lyophilization for the preservation of organisms. Moreover, electrospinning provides an excellent method for encapsulating and orienting biological materials (DNA, proteins, drugs, etc) and organisms.

Keywords: encapsulation, bacteria and viruses, electrospinning

P252: **Evaluation of antibacterial activities of ethanol extract of Saturejabachtiarica**

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Background and Aim: The use of plants as natural food preservatives has special significance. Plant extracts contain several compounds that can be used in food and pharmaceutical products..The present study was conducted to investigate the antibacterial effects of Satureja bactiarica on two foodborn bacteria including , Salmonella typhimurium and Pseudomonas aeruginosa.

Methods: 2 % and 5% concentrations of Satureja Extract were provided in meat extract. 106 CFU/ml of each organism added to experimental trials and incubated at 4 and 15˚C. The number of bacteria counted after 0, 6, 12, 24 and 48 post inoculations.

Results: The results from evaluation of the antibacterial effects of the Satureja bactiarica revealed that at 4 and 15 °C, the growth of , Salmonella typhimurium and Pseudomonas aeruginosa in test tubes containing meat extracts has increased from the beginning to the end of the experiment. In the present of the extracts of Satureja bactiarica at the concentration of 2% and 5%, at 4°C, , the growth of the both bacteria significialy decreased in comparison to time zero and control group ($p<0.05$). Obtained results of this study were shown that growth of Salmonella typhimurium in15C temperature and in concentration of 2 and 5percent of Satureja was significialy decreasd all times in comparison to control group . While the growth of the Pseudomonas aeruginosa was not significialy decreasd at 15 °C.

Conclusion: Based on the results from this study it is concluded that Satureja bactiarica can be used as preservative against these bacteria in the food industry.

Keywords: Satureja bactiarica, Salmonella typhimurium, Pseudomonas aeruginosa, Meat extract.

P253: **Detection of cow raw milk contamination rate by Brucella abortus in Kashan city by Milk Ring Test**

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Background and Aim: Brucellosis is a zoonotic disease caused by gram-negative bacteria Brucella that are pathogenic for a wide variety of animals and human beings. Brucellosis, in particular, is easily transmitted via raw milk. Despite its control in many countries, it remains endemic in Iran. The transmission of brucella from infected animals to humans occurs either by occupational contact or the consumption of contaminated animal products, especially milk, cream and fresh cheese. The purpose of this study was to evaluate the prevalence of cow raw milk contamination by Brucella abortus in Kashan city.

Methods: A total of 50 cow raw milk samples were collected randomly(cluster random sampling) from Kashan suburb milk collection centers from October 2012 to March 2013. Samples were monitored for Brucella abortus antibodies using Milk Ring Test.

Results: The prevalence of Brucella abortus was 8%(4) in Kashan region.

Conclusion: It is concluded from the current study that brucellosis is present in cattle in Kashan suburb farms, where animal breeding is common. Preventive and control measures should immediately and strictly be implemented to protect animals and humans from brucellosis.

Keywords: Milk, Brucella abortus, Kashan, Contamination Rate

P254: **Application of Pulsed-field gel electrophoresis technique in molecular typing of salmonella SPP**

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Background and Aim: Salmonella is a major zoonotic pathogen of food-borne disease throughout the world and is especially common in developing countries. The source of infection in human is contaminated food products . Pulsed-field gel electrophoresis (PFGE) is a golden standard for typing of bacteria such as salmonella species .This technique is also the best choice for an integral subtyping tool used by several national public health networks to differentiate outbreak strain clusters. Therfore , the aim of this study was to determine the frequency a more stable and discriminating genotyping tool is needed to differenciate molecular epidemiologic characteristics within the same Salmonella serotype isolates by PFGE technique.

Methods: All Salmonella isolates were used for this study obtained from Microbial Culture Collection of Microbiology Department, Razi Vaccine & Serum Research Institute, Iran,Karaj.The confluent growth of Salmonell cells were suspended in TE buffer (OD=1.3–1.6 at 600 nm) and then mixed with an equal volume of molten 2% low-melting-point agarose (1: 1) and pipetted smoothly into small plug moulds. The plugs were lysed by incubation an overnight at 50°C in Lysis buffer and 1mg/ml proteinase K. The cell debris and proteinase K were then removed by 4 times washes in 4 ml of TE buffer .The embedded DNA in plugs is digested with Xba I for 3 h at 37ºC and the obtained fragments were separated in 1 % agarose gels using the CHEF system. Electrophoresis was carried out with 0.5X TBE buffer at 6 V/cm and 14 °C. The running time was 22 h and the pulse ramp time was 2.2–52.2 s. Gels were then stained with ethidium bromide and photographed under ultraviolet light.

Results: In order to obtain the epidemiological properties of isolates, PFGE using one enzyme (XbaI) was performed on SE isolates. XbaI was determined to be the more efficient enzyme,The best density for bacterial suspention was fond 1.3–1.6 at 600 nm and the appropriate time for proteinase K effect was overnight.

Conclusion: Infection of human with Salmonella SPP is significant for public health worldwide since Salmonella is the most frequently reported associated with gastroenteritis. These result indicated possible role of contaminated food products as a source of human infection with Salmonella SPP, hence these efforts are needed to eliminate Salmonella from poultry meat & daily food intended for human consumption. The findings of this study, suggest that the most reliable and sutable approach to fingerprinting of Salmonella for epidemiological investigations is PFGE.

Keywords: Salmonella , PFGE, genotyping ,epidemiological study

P255: **Enterotoxin A gene within Staphylococcus aureus isolated from raw milk and traditionally dairy products**

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Background and Aim: S. aureus is a one of the most causes of food poisoning in raw milk and dairy products. Surveillance of Food contamination with S. aureus carrying SE is often difficult. Food processing may kill the bacteria without destroying the thermo-stable SE. The aim of present investigation was to screen different dairy foods collected from Tehran for SE contamination.

Methods: Totally, 120 sample of raw milk (60 sheep's milks, 30 cow's milks and 30 goat's milks) and 100 samples of domestic cheeses were collected from different markets in Tehran city. The samples were then analyzed by routine bacteriological methods for the presence of S. aureus. The isolated bacteria were evaluated by PCR tests for detection of the genes encoding SEA.

Results: The results show that 20% (9 strains) of the S. aureus isolated in raw milk were positive for SEA gene and 25% (2 strains) of the S. aureus isolated from domestic cheeses were positive for SEA gene. This Entertoxin is heat stable, therefore heat has no effect on detoxifying of dairy contamination with enterotoxin.

Conclusion: Our findings suggest that PCR is a rapid, sensitive and inexpensive method for detecting SE and can replace the traditional assays.

Keywords: Keywords: Food poisoning, Staphylococcus aureus, Dairy products, SEA Gene

P256: **Identification of lactic acid bacteria probiotic yogurts Industrial and local industry in Iran**

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Background and Aim: Youghrt is a semi-solid fermented dairy products traditionally produced in Eastern Europe. The role of lactic acid bacteria in the fermentation process of the dairy product is important. Lactic acid bacteria (LAB) fermentation process of carbohydrates to produce acidogenic have the ability to grow in an acidic environment. Some of these bacteria with probiotic properties are also. Probiotics are beneficial microorganisms that plays a protective role in the body do today to increase the quality of food products are added to the food.

Methods: In this study, 30 samples from our 15 local and 15 industrial sample was collected. Both the MRS and M17 in order to increase the sample was selected. Grow at temperatures of 37 and 42 ° C and grown for 24, 48 and 72 hours, respectively. Then fired stages to determine and isolated (LAB) physiological characteristics, morphological and biochemical variables. following the separation of isolates were tested for the separation of probiotics. Isolation and identification of lactic acid bacteria using probiotics to aid kits API50CHL was tolerance test tolerance of bile salts in acidic conditions.

Results: After examining the results of 31 lactic acid bacteria colonies were identified. Variation in local yogurts were higher than industrial sand. The bacteria include Leuconostoc mesenteroides subspecies cremoris, lactobacillus fermentum 1 , like lactobacillus Delbruck subspecies bulgaricus, lactobacillus heloticus and cocobacill includes Pediococcus damnosus and streptococcus thermophilus , through heir biochemical features by api50CHL. Two species of lactobacillus fermentum 1 and lactobacillus heloticcus as the probiotics with high tolerance have been separated in this study.

Conclusion: The results of the research studies conducted by some researchers in this field are aligned. Oylum Erkuş of Turkey, Julia Mavhungu of Africa, Omar Turki, Mamdoh Ershida of Pakistan, Özlen Erdgorul Turkey Ingo, Klara and Guido Werner of America, Ehab Essa Kheadr of Canada, Mebrouk Kihal of Africa, L.Z.jin from Malaysia and Gowri Sukumar of the Indian Research are recorded in the same field.

Keywords: LAB-probiotic-yogurts

P257: **Antibacterial activity of soy protein film incorporated with Zataria multiflora essential oil**

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Background and Aim: Packaging materials such as petroleum synthesized derived plastics have led to adverse side effects on the environment and consumer health. Scientists are suggested to use the safe and biodegradable agricultural materials. Edible films can be prepared from protein, polysaccharides, lipids or the combination of these components. Soy protein was used as safe, low cost, easy availability and biodegradability material for make edible film. There is considerable interest to use essential oils with antimicrobial activities for controlling pathogens microorganisms in foods. The first aim of the present study is to investigate the possibility of formation soy protein film with Zataria multiflora essential oil then determination antibacterial activity of this film agents Staphylococcus aureus ،Lactobacillus plantarum and Escherichia coli.

Methods: Essential oil from Zataria multiflora was incorporated in to the soy protein film solution at six concentrations of 0%, 5%, 10%, 15%, 20%, 25% and 30% (v/v) of edible film. Casting/solvent evaporation method was used for film formation. The antibacterial activity and minimal inhibitory concentration (MIC) was tested by Agar diffusion method.

Results: It is possible to produce soy bean protein film incorporate whit Zataria multiflora essential oil. Escherichia coli and Staphylococcus aureus show maximal and minimal resistance to antibacterial activity of Zataria multiflora essential oil respectively. MIC concentrations of Zataria multiflora essential oil for Lactobacillus plantarum ،Staphylococcus aureus and Escherichia coli are 10, 5 and 15% respectively.

Conclusion: The antibacterial effects of Zataria multiflora essential oil could be considered as preservative materials for foods packaging.

Keywords: Antibacterial activity, Soy protein, Zataria multiflora, essential oil

P258: **Inhibitory effect of pullulan film incorporated with Zataria multiflora essential oil against Lactobacillus plantarum and Escherichia coli**

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Background and Aim: In recent years using biodegradable agricultural materials is so popular in the world. These packaging materials such as pullulan film unlike petroleum synthesized derived plastics don't have any adverse side effects on the environment and consumer health. Pullulan films are odorless, tasteless, colorless, heat-sealable, transparent, highly flexible and low permeability to oxygen and oils. There is significant interest to use essential oils with antibacterial activities for increasing shelf life and controlling pathogens microorganisms in foods. The first aim of the present study is to investigate the possibility of formation pullulan film with Zataria multiflora essential oil then evaluation inhibitory effect of this film against Lactobacillus plantarum and Escherichia coli.

Methods: Essential oil from Zataria multiflora was incorporated in to the pullulan film solution at six concentrations of 0%, 5%, 10%, 15%, 20%, 25% and 30% (v/v) of edible film. Casting/solvent evaporation method was used for film formation. The inhibitory effect of obtained films was determined against Lactobacillus plantarum and Escherichia coli then minimal inhibitory concentration (MIC) was tested by Agar diffusion method.

Results: It is possible to make pullulan film incorporate whit Zataria multiflora essential oil. Escherichia coli was more resistance than Lactobacillus plantarum. MIC concentrations of Zataria multiflora essential oil for Lactobacillus plantarum and Escherichia coli are 10 and 20% respectively.

Conclusion: It is possible to use Zataria multiflora essential oil to make antibacterial films for preservation foods and have inhibitory effect against Lactobacillus plantarum and Escherichia coli.

Keywords: Pullulan, Zataria multiflora, Lactobacillus plantarum, Escherichia coli

P259: **Study of E.coli contamination in frozen meat poultry in Ilam University of Medical Science restaurant**

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Background and Aim: Microbial food safety has been emerged to be a global concern. In response to increasing number of food-borne illnesses, governments all over the world are intensifying their efforts to improve food safety. So that, statistical estimation show that there are approximately 76 million food-borne illnesses each year in the United States that most of these illnesses are undiagnosed and approximately 325,000 cases result in hospitalization and 5,000 are fatal. Among them, nearly 270,000 cases are caused by pathogenic Escherichia coli. Moreover, other implication is transmission of resistant factors from these foods to humans that commonly occur. Therefore, the aim of this study is estimate prevalence E. coli in consumed meat poultry in our university restaurant.

Methods: 24 samples of frozen poultry randomly selected and transmitted to the microbiology laboratory. For the enrichment, 25 g of each sample transferred to the 225 mL tryptic soy broth (TSB) and incubated for 24 h at 37 °C. Then, all specimens were cultured on MacConkey agar plates and the suspected E.coli colonies were sub-cultured on MacConkey agar plates. The final pure cultures were identified by using specific biochemical media for the identification of Enterobacteriaceae. Then, antibiotic susceptibility was determined by disk diffusion method (using antibiotics Piperacillin, Gentamicin, Ciprofloxcacin, Amikacin, Ofloxcacin, Chloramfenicol, Tetracycline, Ampicillin, Cefotaxime and ceftriaxone) and these results compared with human clinical samples that obtained from Ilam hospitals.

Results: In this study, E. coli was isolated from all of the samples that indicated all of them are contaminated. Also, the results of antimicrobial susceptibility test showed that in these isolates antibiotic resistance of the Ciprofloxcacin, Ofloxcacin, Chloramfenicol, Tetracycline and Ampicillin (71.4%, 71.4%, 71.4%, 85.7% and 100%, respectability) in compare with human isolates (40%, 35.3%, 19.3%, 63.3% and 82.7%, respectability) is more. In addition, other species of Enterobacteriaceae, including Proteus myxofaciens (45.8%), Citrobacter murliniae (33.3%), Raoultela planticola (16.6%), Salmonella gallinarum (12.5%) and Proteus vulgaris (8.3%) was isolated.

Conclusion: This study indicates that there are high contaminations in meat poultry. This contamination can occur at multiple steps along the food chain, including production, processing, distribution, retail marketing, and handling or preparation. Aside from this, another implication is transmission of resistant clones and resistance plasmids of these species from poultry to humans that can occur.

Keywords: Microbial food safety, E. coli, antibiotic susceptibility

P260: **Susceptibility Study of Isolated Staphylococcus aureus from organic milk and cheese samples of zanjan city to antimicrobials Drugs**

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Background and Aim: Staphylococcus aureus has been shown as the second leading cause of food born disease. Milk and cheese are the proper substrates for the growth of S. aureus. Overuse of antibiotics have been lead to the development of resistance to multiple antibiotics. The aim of the present study is the Susceptibility Study of Isolated Staphylococcus aureus from organic milk and cheese samples of Urmia city to antimicrobials Drugs.

Methods: 100 samples of cheese and milk were collected randomly from Urmia citymarkets followed by,have cultured in spescific Brad Parker Agar medium then,the bacteriawas isolated. after performing of Gram staining and biochemical tests (mannitol salt agar, catalase, oxidase andCoagulase tests),the bacteria was carried out to Hinton agar and some antibiotic disks were used to determine the sensitivity of bacteria.

Results: Of 100 samples, 44 samples were positive for S. aureusand antimicrobial resistance rates were as follows: Nalidixic acid: 69/07% rifampin 52/30% Gentamicin 49/7%, tetracycline 29/25%, Amoxicillin 12/69%, nitrofurantoin 2/7%.

Conclusion: The results were showed that the majority of isolatedS. aureuswere resistant to one or more antimicrobial agent, in which the highest percentage of antimicrobial resistance was to nalidixic acid.

Keywords: Keywords: Staphylococcus aureus , Milk and Cheese , sea gene , PCR.

P261: **Isolation and Detection of Entrotoxogenic Type A Staphylococcus aureus from organic Dairy in Zanjan by PCR.**

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Background and Aim: Staphylococcus aureus is a facultative anaerobic gram-positive coccus ; it is non-motile and catalase and coagulase positive. Cells are spherical single or paired cocci, or form grape-like clusters. S. aureus is one of the major human pathogens and is the most frequent causes of food poisoning in dairy products. The main etiologic agents of food poisoning are staphylococcal enterotoxins. Milk and cheese are good substrate for S. aureus.

Methods: In this study we have detected S. aureus in 44of 100 samples of organic milk and cheese which have collected from food stores in Zanjan, Iran. The bacteria from milk and cheese samples were isolated on Baird-Parker agar. Following the biochemical tests , the PCR reaction has been done to identify the bacterial colonies. The bacterial colonies which have been positive , have checked for sea g ene using specific primers

Results: . The results have shown that 44% of samples were contaminated with S. aureus . The S. aureus colonies have been checked for sea gene and only 13samples have contained sea gene.

Conclusion: In this research ,sea gene specific PCR showed promising result in differentiation of enterotoxigenic and non-enterotoxigenicisolates of S. aureus in organic milk and cheese samples.

Keywords: Staphylococcus aureus , Milk and Cheese , sea gene , PCR

P262: **Study on the prevalence rate of coagulase – positive staphylococcus aureus inlocal cheese villages suburbs. Zanjan**

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Background and Aim: Staphylococcus aureuse as the third important factor in diseases of food origin are discussed . Milk raw material sutiable for the growth of Staphylococcus aureuse and dairy products, the source is documented for Staphylococcal poisoning.

Methods: This study determind the prevalence Staphylococcus aureuse local chesse in envirens villages Zanjan by techniques has been done. Total 103 samples randomly selected collected form villages suburbs.

Results: Based on the culture methed 11/6% of on samples infected with Staphylococcus aureuse 11/6 %of on samples infected with Staphylococcus intermediuse findiny indicates that 23/3% of samples infect with Staphylococcus coaglase positive 77/8% of samples infected with Staphylococcus coaglase negative.

Conclusion: This necessitates attention observation concerning hygienic condition during production and requires the use of pasteurized milk to produce cheese.

Keywords: local cheese , Staphylococcus aureuse , culture

P263: **Comparison of microbial contamination of meat products in three state of cooked, raw and half-baked ready for sale in the city of Marand - Iran**

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Background and Aim: Despite advances in the technologies of Food Science and industry, diseases related with cooking of food has remained as the most important issue in the world. Around the world hundreds of millions of people suffer from diseases transmissible through food and water. The problem in developing countries and among people who have weakened immune system or suffering from malnutrition is growing. During the past decade, there was a significant increase in reports of food borne disease. This study evaluated the microbial contamination of ready for sale meat products (barbecue, hamburgers ...) in three state of raw, cooked and half-baked

Methods:: During 10 months, 390 samples including 130 samples of raw kebabs and hamburgers,l10 samples of half-baked kebabs and barnburners and 160 samples of cooked kebabs and barnburners from several units at a tie and randomly were sampled and were sent to the laboratory at standard conditions The samples based on Iran standards were analyzed.

Results:: Considering the microbiological tests, all cooked samples of bacterial contamination is in standard range and was therefore usable. But from 130 samples of raw kebabs 99 samples were consumable (76.15%) and 31 samples were not consumable (23.85%) and from 110 samples of half-baked kebabs, 102 samples were consumable (92.72%) and 8 samples were non-consumable: (7.28%)

Conclusion:: The results of this study show that consumption of cooked meat and hamburgers will be no concern for consumers. It also recommended that consumptionof meat products, raw and half-baked to be avoided.

Keywords: microbial contamination - meat products - Comparison

P264: **Prevalence of Listeria monocytogenes in raw milk of Qom**

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Background and Aim: Listeria monocytogenes is an important pathogen that is transmitted via food and can make in human a variety of diseases such as meningitis, septicemia, etc., These bacteria are Gram-positive, non-spore and Because tolerate high concentrations of salt, extreme temperature and pH changes can often food sources such as milk, cheese and meat to be found And because a lot of concern for livestock products is dependent on the food industry So in the cycle of milk production and distribution should be done control to prevent of the infection can be exerted at a high level....

Methods: In this study, the contamination of raw milks obtained from farms in different parts in qom to Listeria monocytogenes was studied. For this purpose, of the winter of 1389 to the autumn of 1390, 100 caws in the city of Qom randomly selected and 100 samples of milk were collected. Initial microbial load of samples was determined and each sample in Listeria enrichment medium and then were cultured in Oxford. Suspected colonies by doing differential tests, such as SIM, bile esculin, glucose, lactose, etc. for identification of Listeria monocytogenes were examined

Results: Microbial load of milk samples collected and compare it with Iran milk microbiological standard showed that 84% of raw milk samples excellent grade, 14% were grade 1 and 2% of cases were grade 2. on The other of 100 raw milk samples, 9 samples gram-positive bacil, catalase positive, oxidase negative, were suspected of Listeria monocytogenes. Differential tests results as hydrolysis of esculin positive, glucose positive, lactose positive, MR positive, VP negative, an umbrella movement started in the SIM medium at 22 ° C, urease negative, negative indole production,negative nitrate reduction, citrate negative, D- xylose negative, L-rhamnose positive and result of the A / A in the TSI medium, Listeria monocytogenes contamination of the samples confirmed. So the 9% of samples of contamination of listeria monocytogenes were positive.

Conclusion: identification and treatment of infected animals for improving health of Dairy products, especially milk , is very important.

Keywords: listeria monocytogenes- raw milk- qom

P265: **Anti-aflatoxigenic activity of natural phenolic compounds by suppression of significant genes involved in aflatoxin biosynthesis pathway in Aspergillus parasiticus NRRL 2999**

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Background and Aim: Aflatoxins (AF) are a group of polyketide-derived secondary metabolites produced mainly by certain strains of the common molds Aspergillus flavus and Aspergillus parasiticus. For food safety control and to protect consumers from harmful contaminants, the presence of toxigenic fungi must be checked at critical control points during production of agricultural commodities as well as during the process of food and feed preparation. Therefore many natural compounds found in dietary plants, such as extracts of herbs and fruit extracts, possess antimicrobial activities against Aspergillus parasiticus. In the present study, we use curcumin and eugenol as antioxidant compounds with the aim of finding novel and safe AF inhibitor, with natural base, which can be used for controlling AF contamination of food and agricultural commodities.

Methods: Aspergillus parasiticus NRRL 2999 was cultured in presence of different concentrations of curcumin (125 to 2000 µg/ml) and eugenol (15.62 to 500 µg/ml) in YES medium for 3 days at 28°C. The fungal mycelia was separated from the culture medium and the total fungal weight was determined by drying at 80 ºC for 2 hours. AF production was determined by HPLC. The expression of ver-1, nor-1, pksA, omtA and aflR genes in aflatoxin biosynthesis pathway was evaluated by real time PCR.

Results: AFB1 was inhibited in the range of 15.1% to 97.1% and 26.6% to 94.9% by serial two-fold concentrations of eugenol and curcumin, respectively. Maximum inhibition of fungal growth was reported around 95.8% and 60.8% for the highest concentration of eugenol (500 µg/ml) and curcumin (2000 µg/ml), respectively. Analysis of the expression of aflatoxin pathway genes by real time PCR showed that these compounds inhibited the expression of all tested genes i.e. ver-1, nor-1, pksA, omtA and aflR.

Conclusion: The significant reduction of growth and aflatoxin B1 synthesis by natural phenolic compounds in the present study suggest that these compounds may be employed successfully in controlling of fungal growth and subsequent aflatoxin contamination of food and feed.

Keywords: Aflatoxin, Aspergillus parasiticus, Curcumin, Eugenol, Aflatoxin inhibitor, HPLC, Real-time PCR

P266: **Study of Probiotic strains properties and survey of their functional and health effects (in vivo & in vitro)**

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Background and Aim: Probiotics are live microorganisms which when administered in adequate amounts confer a health benefit on the host. Probiotics may beneficially affect the host by augmenting its intestinal microbial population beyond the amount already existing, thus possibly inhibiting pathogens. Lactic acid bacteria (LAB) and bifidobacteria are the most common types of microbes used as probiotics, but certain yeasts and bacilli may also be used. Probiotics are commonly consumed as part of fermented foods with specially added active live cultures, such as in yogurtor as dietary supplements. Recently, research on probiotics has expanded rapidly. The main target of this research was to establish these bacteria in the digestive organs without medical intervention as an alternative to antibiotics and reduces of treatment costs.

Methods: Interactions between probiotics and other ingredients could happen and those interactions can be protective, neutral, or detrimental to probiotic stability. Properties of probiotics on human health can be done either directly in the population (in situ) or tests by simulated conditions (in vitro) and also studies on the animals.

Results: Health effects of probiotics may result from the activity of living and non-living cells or their metabolites. Intestinal microbial balance, improves lactose intolerance, symptoms and treatment of diarrhea, improved sensitivity or allergy, improved nutritional value, anti-mutagenic, anti-cancer, prevention of infection, prevention and treatment for women. Stimulate, promote and regulate the immune system, boosting activity Phagocytosis stimulate cytokine activity, decreased serum cholesterol among the most important issues in the field of probiotics therapy are discussed.

Conclusion: This study assessed the health effects of probiotics and analyzes the treatment process that due to the increased use of probiotic products can be helpful.

Keywords: Probiotic strains, functional effects, health effects

P267: **Biochemical and molecular characteristics of Listeria ivanovii isolated from sheep meat**

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Background and Aim: Listeria is the causal agent of listeriosis which is a foodborne disease. Two species of Listeria are pathogenic, Listeria monocytogenes and Listeria ivanovii. The role of Listeria ivanovii is important in abortion, stillbirth, septicaemia in sheep and cow's infections and sometimes is pathogenic in humans. Contamination of ovine carcasses during the sluagther and processing is a major risk for foodborne infection in humans. Considering the importance of what mentioned, the purpose of this study was to detect Listeria ivanovii in sheep meat in Shahrekord area.

Methods: A total of 200 samples of sheep meat were collected from Jooneghan abattoir, Chaharmahal va bakhtiari province. samples were enrichmented by use of two enrichment method, Listeria enrichment broth as primary enrichment and Frazer secondary broth as secondary enrichment, and then subcultured on PALCAM agar. After doing specific biochemical tests we did PCR for identification of Listeria spp.

Results: According to the characteristics of the colonies morphology, biochemical tests and PCR method. Five isolates (2.5%) were recognized as Listeria ivanovii.

Conclusion: Our results showed that 2.5% of slaughter sheep meat in Shahrekord area were contaminated with Listeria. Although the contamination rate in sheep carcasses with pathogenic L.ivanovii was low, but the role of red meet in transmission thise important foodborne disease should be considered.

Keywords: Listeria ivanovii, Biochemical characteristics, sheep meat

P268: **Prevalence of Listeria spp. in Fish shops in urmia city (North West of Iran)**

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Background and Aim: The present study was conducted to determine the prevalence of Listeria spp. in fish obtained from Urmia fish markets.

Methods: A number of 194 fish comprising Oncorhynchus mykiss (n=42), Sander lucioperca (n=38), Cyprinus carpio (n=30), Hypophthalmiachthys molitrix (n=48), Abramis brama (n=12), Astacus leptodactylus (n=12) and Silurus glanis (n=12) were obtained from different fish markets of Urmia from June 2009 to February 2010. Listeria isolation was performed in two stages including enrichment in cold and selective plating. After colony formation, in order to confirming the genus of the Listeria, a fragment of Prs gene using Listeria genus specific primers was amplified from isolated bacteria using polymerase chain reaction (PCR).

Results: Results revealed that Listeria was isolated from 24 fish (12.37%). The highest prevalence of Listeria was observed in both A. brama and A. leptodactylus with 25%, while the lowest prevalence of Listeria was seen in S. lucioperca (9.7%). From the total of 24 Listeria isolates, five isolates (21%) were confirmed to be L. monocytogenes; seven isolates were L. ivonoi (29%) while L. Seeligeri was not isolated from any examined fish.

Conclusion: The study showed that L. monocytogenes and other Listeria species are common contaminant of fish obtained from Urmia fish markets, and this may pose serious public health implications.

Keywords: Listeria, Listeria monocytogenes, fish

P269: **Modelling of fermentation time and rheological properties of symbiotic yogurt containing lactobacillus reuteri using response surface methodology**

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Background and Aim: Yogurt products have been used as the good vehicle to incorporate probiotic microorganisms to consumers. The use of probiotic bacteria in yogurt is increasing because of their therapeutic effects. As well as, since textural and rheological properties of yogurt play an essential role in quality and consumer acceptance, several investigations have focused to improve low-fat yogurt properties by incorporation different prebiotics.

Methods: Response surface methodology was employed to investigate the combined effects of probiotic inoculum level (1-3% v/v), fermentation temperature (37-45˚ C) and plant sterol ester addition (0-1% w/v) on fermentation time and rheological properties of symbiotic yogurt. The rheological characteristics were measured by dynamic oscillatory rheometery.

Results: The second-order polynomial model was fitted to the fermentation time, structure strength (A value), resistance to mechanical force or yield stress (τy) and complex viscosity (η*) of runs as the responses. Analysis of variance revealed that the quadratic models are well adjusted to predict the experimental data. Lack-of-fit tests were insignificant and determination coefficients (R2) were higher than 89.3%. The results showed that fermentation time decreased with increasing plant sterolester content, incubation temperature and probiotic inoculum level. Rheological properties significantly influenced by independent variables. Medium levels of probiotic inoculum resulted in stronger gels. Plant sterol ester addition had a softening effect on yogurt, a positive effect of incubation temperature on structure strength was also obvious.

Conclusion: Therefore, to gain all purposes (nutritional properties, economic aspect and acceptable texture), the optimum level of probiotic inoculums, fermentation tempreture and plant sterol ester should be considered.

Keywords: Yogurt, Rheological properties, Response surface methodology, Plant sterol ester, Probiotic

P270: **Evaluation of antibacterial effect of mango kernel extract against Pseudomonas aeroginosa**

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Background and Aim: The mango seed kernel is believed to possess antibacterial effect against different bacteria. In the present study we evaluate the antibacterial activity of mango seed kernel on Pseudomonas aeroginosa which is clinicaly important.

Methods: Two types of mango were purchased from the market. The mango seed kernels were dried and the ethanol and acetone extract of seed kernels were prepared prior to the antibacterial evaluation. Antibacterial activity of mango seed kernel was evaluated on Pseudomonas aeroginosa strain PTCC1599. Pseudomonas aeroginosa was first identified by conventional biochemical methods. Antibacterial effect of mango seed kernel was performed using agar well diffusion methods and minimal inhibitory concentration was determined for both ethanol and acetone extracts.

Results: Pakistani type of mango showed the highest antibacterial effect on Pseudomonas aeroginosa. The diameter on inhibition zone of ethanol extract of Pakistani type was about 16 mm. Moreover, ethanol extract of mango seed kernel more effectively inhibited growth of Pseudomonas aeroginosa. Along with increasing the concentration of extracts, the diameter of inhibition zone also increased.

Conclusion: Our results showed that both ethanol and acetone extract of mango seed kernel can effectively inhibit growth of Pseudomonas aeroginosa. As regards, mango seed usually wasted during food processing and these process lead to accumulation of hundred tons of waste in the environment, we strongly suggest using mango seed kernel in cosmetics, food processing and other related industries.

Keywords: Mango, Pseudomonas aeroginisa, food processing, ethanol extract

P271: **Oxidative Damage Caused by Toxically Bacteria in Milk**

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Background and Aim: Lipid peroxidation is one of the increasingly undesirable in the food industry that could adversely affect the taste, smell and color of food. Furthermore, this process leads to the production of harmful and toxic compounds in food intake will lead to complications and disease. Escherichia coli is a Gram-negative bacterium that is found in the digestive tract of animals and subsequently, the food of animal origin from different ways may become contaminated. Present study was to evaluate oxidative damage and lipid peroxidation is designed for aerobic bacteria in the milk.

Methods: First, 1 mL of milk is added to a test tube. Standard E. coli (ATCC 25922) in dilutions (105 106 107) was prepared and added to the test tube. The samples were incubated at 37 ° C for 20 h. The bacteria damage the lipid peroxidation was assessed by testing T-bars. After bacterial growth, Damage of lipid peroxidation was assessed by T-bars test.

Results: Lipid peroxidation in the group with high levels of bacteria (107) had received significantly increased. Lipid peroxidation levels in this group was 0.025 ± 0.01, compared to the control group, that was 0.009 ± 0.01.

Conclusion: Bacteria addition to cause infection, the role in the lipid peroxidation that is uncorrectable in the process, will also. So serious measures to prevent contamination of food -sensitive, such as foods of animal origin, must always be observed.

Keywords: Escherichia coli - Lipid Oxidation – Milk

P272: **Evaluation of Microbial Quality of Ice Creams In Tehran**

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Background and Aim: Ice-cream, a milk based product, due to comprising rich ingredients, almost neutral pH value and storage duration, can be a suitable and enriched media for microbial growth, which can cause food poisoning among consumers. So the microbial quality of it should has been carefully considered to ensure public health. The assessment of microbial quality of produced and sold ice creams in Tehran was the aim of this study.

Methods: This study was done between years of 1387 to 1390 in microbiology laboratory of food & drug administration of Tehran University of Medical Science. Accessed the microbial quality of 94 pasteurised and 140 unpasteurised sold ice creams in Tehran stores.Under the national Iranian standard no: 2406: milk & dairy product microbiological specifications condition tests for total micriorganism and Enterobacteriaceae count & detection of presence of Escherichia coli, Staphylococcus aureus,mold, yeast and Salmonella spp were done. Analysing the final data was done by the spss software and using Statistical formulas and also $\chi 2$ and t-test.

Results: Generally, total microbial contamination was seen in 45.74% (p=0.15) of pasteurised samples and 97.14% (p=0.0005) of unpasteurised samples beyond the standard limits. Bacterial contaminations by Enterobacteracea were seen in 50% (p=0.55) of pasteurised samples and 99.28% (p=0.01) of unpasteurised samples,and by Escherichia coli were seen in 19.28% (p=2.7) of unpasteurised samples,and by Staphylococus aureus were seen in 5.7% (p=2.2) of unpasteurised samples, and by total mold and yeast were seen in 38.29% (p=2.1) of pasteurised samples and 64.28% (p=1.5) of unpasteurised samples. The presence of E. coli and S. aureus was not seen in pasteurised samples. Also no Salmonella spp was isolated in any of the samples tested.

Conclusion: Ice-cream must include extremely low bacterial load. Observation of E. coli bacteria indicates the fecal contamination. The presence of S. aureus due to staphylococcal food poisoning because of staphylococcal enterotoxins in the food. As the results were, total microbial contamination and Bacterial contaminations by Enterobacteracea of unpasturiezead ice cream samples being sold in Tehran are concerned and these shown the public health should be taken care of food borne diseases. So there should be stronger supervision and even commitment by regulatory agency to remove the risk of pathogens into the public health.

Keywords: Microbial Contamination, E. coli , S. aureus, Enterobacteracea, ice cream.

P273: **Antimicrobial functionality of nanofibers made from food hydrocolloids and their application in active packaging**

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Background and Aim: Electrospinning is an ultrathin fiber forming method that uses electrostatic force to make polymer solution into fine fibers. These nanofibers have been explored for use in applications such as drug delivery, affinity membranes and recovery of metal ions, wound dressing, tissue engineering scaffolds, and biosensors; however food-related purposes is relatively new. One form of active packaging relates to incorporation of antimicrobial agents which are allowed to diffuse into the product to hinder survival of microorganisms during storage time. Electrospun fibers may offer a novel active packaging trend compared to film and sheet carriers, such as being more responsive to changes in the surrounding atmosphere like relative humidity and temperature changes, furthermore because the electrospinning process takes place at ambient conditions, electrospun fibers are more suitable for encapsulating thermally-labile active agents like Nisin and Natamycine or plant essential oils . While many synthetic polymers can be electrospun readily when dissolved in organic solvents, but they could not be used safely as a controlled release trend in foods. Hydrocolloids such as gelatin, pectin, alginate, poly lactic acids and cellulose derivatives can be a potent alternative for production of these fibers. Since many of these biopolymers are food grade and they are generally recognized as safe (GRAS) in the food industry, so they could be used as functional ingredient in food contact materials. Electrospun biopolymer fibers are considered a reliable carrier/delivery device for many bioactive agents. In this work we discuss about the issue of producing nanofibres by means of food grade hydrocolloids containing natural antimicrobial agents and how process parameters of electrospinning affects controlled release of these natural preservatives

Methods: At first different concentration of hydrocolloids has been prepared to meet the viscosity ,conductivity and protecting capability of antimicrobial agent shell material .Two methods, direct and indirect addition, were used to incorporate antimicrobial agent like Allyl Iso thiocyanate (AITS)or Nisin prior to spinning the fibers. Most common polymeric base solution is PLA (poly lactic acid).Then fiber forming solution was driven to electrospinning setup by a syringe pump. High voltage up to 25-30 KV was applied to the solution coming from needle and finally fibers are made. Different SEM pictures and release kinetic studies are necessary to establish entrapment of bioactive agent within nanofibers web.

Results: Different publishes revealed that as concentration of antimicrobial agent increased, the fibers progressively became smaller in diameter with a concomitant formation of beads. In comparison, the dilution of PLA-fiber forming solutions resulted in fibers of smaller diameter and with more tapered beads but no fiber-to-sphere transition. Antimicrobial agent release electrospun fibers can be controlled by varying the relative humidity.

Conclusion: Electrospinning enables the formation of fibers having diameters of less than 100 nm and therefore can exhibit nano effects. Studies demonstrate that developing new biopolymer fibers through electrospinning is an emerging field in intelligent packaging. Polymers also improve their properties and can enhance their spinnability. Antimicrobial agent release from electrospun fibers triggered by moisture appears to be a promising carrier for this antimicrobial compound in active packaging applications.

Keywords: Electrospinning, nano fibres , active packaging

P274: **Introduction of some physicochemical measurements for detection of conventional adulteration of milk**

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Background and Aim: One of the commonly adulterated foods is milk which may contain chemical additives such as sodium carbonate, sodium bicarbonate, formalin, starch, urea, detergent, glucose, sodium chloride, ammonium sulfate, benzoic acid etc. These are added to milk as neutralizers to increase its shelf-life and to prevent curding. Long-term consumption of the adulterated milk may cause to health hazards. Some rapid measurements using sophisticated tools are introduced for estimation of bacterial cell number into the milk based on the physicochemical parameters such as electrical conductivity, impedance or electrical resistance. As metabolizingmedium substrates, uncharged or weakly charged large molecules such as proteins, carbohydrates and fats are transformed to smaller highly charged end products, such as amino acids, lactate and acetate, thereby causing significant changes in electrical impedance, conductance and capacitance of the medium. We studied the effects of conventional adulteration of milk on total bacterial count and physicochemical characteristics of growth media.

Methods: Natural milk and adulterated milk samples were prepared. Adulterated milk samples were contained: natural milk + (NaCl, sodium carbonate, starch, urea, detergent and benzoic acid). Physicochemical parameters and the number of viable bacterial cells in milk before and after adulteration were investigated.

Results: The number of bacteria did not changed significantly in adulterated milk, however, significant difference were observed in the electrical potential difference, conductivity (Con), pH, resistivity (Res), total dissolved solids (TDS), salinity (Sal), in the case of NaCl, sodium bicarbonate, starch, urea, detergent and benzoic acid.

Conclusion: By addition the studied adulterant agents to milk, microbiological quality of milk does not change, while there were significant changes in physicochemical parameters such as electrical conductivity and resistance. Therefore it is suggested to screen the milk samples for being adulterated when quality control methods are based on physicochemical change of the milk during the incubation. Adulteration in milk can led to the negative false during the screening of samples by standard plate count method. However, these adulterations can be measured easily and rapidly by the commercial conductivity meters.

Keywords: milk- Adulteration- Physicochemical parameters

P275: **Novel Heat stable Single Peptide Bacteriocins Produced by L.brevis LB32 and L.pentosus LP05 Isolated from Ewes Milk in Iran**

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Background and Aim: Lactic acid bacteria (LAB) play an essential role in the majority of food fermentations, and a wide variety of strains are routinely employed as starter cultures in the manufacture of dairy, meat, vegetable, and bakery products. Many lactic acid bacteria (LAB) produce a high diversity of different antibacterial substances which are defined as bacteriocins or bacteriocin like substances (BLIS) (Cintas et al., 2001; Jose et al., 2007; Zhang et al., 2009). In this study our aim was to investigate the properties of Lactic acid bacteria present in these traditionally made dairy samples made from ewe's milk. We analyzed the antimicrobial spectrum and physico-chemical properties of bacteriocin or bacteriocin like substances (BLIS) produced by these locally isolated LAB strains.

Methods: In this study traditionally made yogurt and sour buttermilk made from ewes milk, were screened for the presence of Lactic acid bacteria (LAB) with probiotic potential.Using 50CHL API system, these isolates were identified to species level. Well diffusion assay was used for determing the antibacterial spectrum of the isolates against pathogens. The antibacterial protein was isolated from the isolates and partially purified and characterized.

Results: Among 77 isolates identified as LAB, ten isolates showed wide range antibacterial spectrum and were inhibitory towards Listeria monocytogenes, Salmonella enteritidis, and Staphylococcus aureus. Using 50CHL API system, these isolates were identified as Lactobacillus pentosus, Lactobacillus brevis, Lactobacillus paracasei (two strains), Lactococcus lactis (two strains) and Pedicoccus acidilactici (four strains). Among the identified isolates, the antimicrobial activity in the supernatant fluids of L.brevis LB32 and L.pentosus LP05 remained unaffected by pH neutralization and catalase treatments, while strongly sensitive to the action of proteolytic enzymes used. The peptide antagonistic compounds produced by L.brevis LB32 and L.pentosus LP05 were heat resistant and were able to tolerate 100°C and 121°C. An enhanced activity (AU/ml) and yields were observed after partially purifying the concentrated culture supernatant fluids of the mentioned isolates by using ammonium sulphate (80%) and DEAE cellulose columns. The approximate molecular weight of the antagonistic compounds as determined by ultrafilter membranes and SDS-PAGE analysis revealed a peptide band of approximately 4.5 and 6 KDa in L.pentosus LP05 and L.brevis LB32, respectively. In contrast to L.brevis LB32, L.pentosus LP05 harbored an 18Kb plasmid DNA which appeared to be carrying the bacteriocin gene as evident by plasmid curing experiments. All the mutants retained their host immunity and were resistant to the bacteriocin produced by the parent strain.

Conclusion: To conclude, there is a wide variety of traditional dairy products especially in rural areas of Iran. These products mostly made from unpasteurized ewes or cows milk are being appreciated by local people regarding its proven health benefits. This study proved the presence of viable probiotic LAB micro flora in these products. The antagonistic activity possessed by these isolates might be used for the control of unwanted pathogens mainly in dairy products, and could be exploited further for use in fermented dairy products.

Keywords: Antagonistic activity, Bacteriocin like inhibitory substances, Ewe milk, Lactic acid bacteria

P276: **Microbial contamination of traditional ice cream in Arak**

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Background and Aim: Ice cream is a nutritious dairy products and high nutritional value and ability to shelf life long is suitable for microbial growth. Since production and distribution this product in traditional centers is particularly high in warm seasons so preparation and distribution in the pasteurization conditions and personal hygiene is not suitable . therefore this study to evaluate the microbial contamination of traditional ice cream was in Arak.

Methods: In this study 165 samples of ice cream manufacturing and distribution centers in Arak in the months of June till September in years 2010 to 2012 selected randomly and according to Iran's national standards for microbial contamination levels were tested.

Results: In this survey was shown that in the year 2010 of 58 samples of traditional ice cream 49(84.48%),35(60.4%) and 7(12.6%) of the samples infected with family Enterobacteriaceae,Escherichia coli,Staphylococcus aureus respectively.. that in the year 2011 of 50 samples 43(56%) infected by family Enterobacteriaceae,18(36%) infected by Escherichia coli,7(14%) infected by Staphylococcus aureus. in the year 2012 of 57 samples sequenced 53(92.98%),29(50.58%) and 6(10.52%) of the samples infected with family Enterobacteriaceae,Escherichia coli,Staphylococcus aureus respectively. In general In this research was shown that during the years 2010 to 2012 of 165 samples traditional ice cream 148(89.69%) of the samples had microbial contamination,145(87.82%) of the samples with family Enterobacteriaceae were known to infected.82(49.69%)of the samples contaminated with Escherichia coli and 20(12.12%)of the samples were contaminated with Staphylococcus aureus. All samples were negative for Salmonella.

Conclusion: The results of this study show the high of microbial contamination in traditional ice cream, Which indicates poor hygiene and unsanitary situation Preparation Centers of traditional ice creams in Markazi province. Because of the importance of the traditional ice cream in the transmission of microbial infections and In order to prevention of food poisoning outbreak due to use of ice cream ,sanitary control of ice cream production and distribution centers and personal hygiene is required in these centers.

Keywords: traditional Ice cream, Microbial contamination, Arak

P277: **The novel biofilm control strategies in food plant**

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Background and Aim: Biofilms are bacterial films consisting of a mixture of different microorganisms, along with product residue and polysaccharids excreted by the microorganisms that help them to attach to surfaces. Biofilm can establish themselves on almost any surface in food production environment.

Methods: In food processing plants, surfaces are periodically cleaned and disinfected, the temperature is generally low or the atmosphere is dry. Thus, the process of biofilm formation is periodically interrupted. Pathogenic microorganisms- such as Listeria and Salmonella- can attach to and grow on food surfaces, equipment and processing environments to form biofilms. Since biofilms are a great concern in the food sectors, many studies have been done in order to gain a better understanding control strategies. The first and most important things to do are (i) disinfection “in time”, before biofilm develops, (ii) disinfection of biofilms using harsh disinfectants, and (iii) inhibition of attachment of microbes by selecting surface materials that do not promote attachment or by supplementing with nutrients.

Results: In this paper, we examine conventional control strategies with chemical-based, as well as potential green biofilm control, including control using enzyme, phage, ultrasonication and microbial interactions and metabolite molecules.

Keywords: Biofilm, Sanitation, control strategies, Conventional, green technology

P278: **Investigation of toxin production genes of Bacillus cereus isolated from raw milk in silo tank**

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Background and Aim: Bacillus cereus as one of the most important gram positive spore former organism causes special problems in dairy industry. Sporeforming and psychrotrophic properties enable B.cereus to survive during pasteurization as well as to grow in milk at refrigerated storage conditions. B.cereus spores attach to the surface and form biofilms. Enterotoxins; cereulide, hemolysin and cereolysin are known as potential virulence factors of B.cereus. It can cause milk product spoilage by lipase and protease. Hence, the purpose of this study was to investigate the biochemical characteristics, lipase and protease production and hemolysin and cereolysin genes contents of B.cereus.

Methods: These were isolated from raw milk and surface of silo tank in 30 days at three dairy plants during winter from North of Iran. MYP agar was used to detect B.cereus. For sampleing of surfaces, the swab test was used.

Results: The results showed that total microorganisms, aerobic spores and B.cereus of raw milk are almost far greater than the US standard in mentioned dairy plants. Positive swab tests indicate that mesophilic B.cereus is common in silo tanks. The presence of mesophilic strains showed that the cleaning system may not be satisfactory. In addition, bacterial cells remained on surfaces can form biofilm. Biofilms could release into the silo milk and couse the high level of microorganisms. Biochemical analysis showed 26% of B.cereus isolates can grow in 10% NaCl. Moreover, 27.7 and 28.3% of isolated were lipolytic and proteolytic, respectively. Although PCR analysis showed that all strains contained cerA gene while cerB and HBL genes were present in 16.3% and 20.4% of strains, respectively. According to the sequenced results of the hemolysin and cereolysin gene, in dairy A, some homogeneous strains were found in raw milk and surface of silo tank.

Conclusion: It was concluded that the equipment surfaces play an important role in contamination of raw milk with B.cereus, especially if hygiene procedures are not applied correctly.

Keywords: Bacillus cereus, raw milk, biofilm, Hemolysin, Cereolysin

P279: **Survival and Growth of Bacillus cereus spores in UF-Feta cheese**

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Background and Aim: Bacillus cereus as a Gram-positive, spore-forming bacterium is widespread in the environment. The spores survive pasteurization and psychrotrophic strains of it limit the keeping quality of milk products. B.cereus produces several toxins, including emetic toxin and diarrhoeal enterotoxins. Hemolysin BL (HBL) and Cereolysin AB proteins are considered as two virulence factors in B.cereus diarrhea. This microorganism possibly occurring in dairy products like UF-Feta cheese as a common milk product use in Iran. In this investigation, we have examined survival and growth of B.cereus as a contamination in UF-Feta cheese.

Methods: UF-Feta cheese from three dairy plants was analyzed for total microorganisms, aerobic and B.cereus spores at three parts of cheese (center, surface and water) during shelf life and the isolates were characterised with respect to their ability to produce HBL, CerA and CerB by PCR.

Results: The results show that total count increased after warm storage, whereas spore and B.cereus count decreased. In spite of decreasing significantly ($p<0.05$) total count after 1st, 2nd, 3th months, the variation of B.cereus and spore count during these months wasn't meaningful. In addition, total count in the center of cheese was greater than other parts, but spore and B.cereus count was greater in surface. Microbial contamination showed increasing during shelf life in water of chasse. In the detection of toxin genes, all B.cereus isolates were found to carry the CerA gene, while 44% and 58% strains were positive for CerB and HBL genes, respectively. According to the sequenced results, two new strains were found in our study not reported in NCBI.

Conclusion: In conclusion, B.cereus strains isolated from cheese samples should be regarded as potential enterotoxin producers according to PCR results. Therefore, new attempts should be undertaken to eliminate these organisms during cheese manufacturing for food safety and quality.

Keywords: UF-Feta cheese, Bacillus cereus, Hemolysin, Cereolysin

P280: **Determination of parameters affecting on antimicrobial activity of Enterococcus faecium**

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Background and Aim: Antimicrobial peptides (AMPs) of Lactic Acid Bacteria (LAB) have potential for use as food biopreservatives to control spoilage and pathogenic bacteria such as Listeria monocytogenes. Some of the Enterococci, a member of the LAB, are found to produce antimicrobial agents such as antimicrobial peptides – the bacteriocins. The aim of this study is to investigate parameters which affect on the activity of AMPs by Enterococcus faecium.

Methods: Bacterial strains, culture media and growth conditions: Enterococcus faecium strain 108 and 124 (the producer strains) were isolated previously from vaginal swabs. The strains were propagated in MRS (deMan, Rogosa and Sharpe) broth at 37°C for 22 h. Listeria monocytogenes was selected as indicator organism. Subculture of producer strains: After 18-24h of incubation, culture media containing the producer strains were centrifuged (12000×g, 10min, 4°C) and the pellets were resuspended, separately. One half of the resuspended pellet of each strain was inoculated in fresh MRS broth, and the other half was inoculated in fresh BHI broth. The prepared samples were incubated for 22 h at 37°C .Preparation of cell-free supernatant: After 22 h of incubation, the culture media were centrifuged and the supernatant fluids were filter sterilized (0.45 μm pore size cellulose acetate filter). Bacteriocin production assay (antimicrobial activity assay): Well diffusion assay and spot on lawn assay were used to detect bactericin production and antimicrobial activity. The assays were performed on BHI agar plates.

Results: The antimicrobial activity of E. faecium strain 124 was compared using well diffusion assay in both MRS and BHI broth of subculture media via comparison of inhibition zone radius. Larger inhibition zone has caused by sample subcultured in MRS than BHI. But higher activity was observed in BHI than MRS broth when strain 108 was subcultured. On the other hand, spot on lawn assay of cell-containing supernatant showed the inhibition zone for both 108 and 124 strains.

Conclusion: Based on our results, we concluded that probably, at least three parameters have affected on antibacterial activity of enterococcus: a) the type of growth media of enterococcus before antimicrobial tests, b) the type of producer strain, c) presence of attacked bacterium. In the other words, better growth inhibition of L. monocytogenes (indicator strain) by enterococcus (producer strain) has been observed in the presence of both producer and indicator strain. Moreover, suitable culture medium may differ from one producer strain to another. Further studies are recommended to elucidate more details.

Keywords: Antimicrobial peptides, antimicrobial activity, culture medium

P281: **Antimicrobial Activity of Pomegranate Flowers (Punica granatum L.) against Food-Borne Disease**

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Background and Aim: Food-borne diseases are taking thousands of lives every year. According to FDA 45 million people become food-poisoned each year among those 128000 are admitted to hospital and 3000 pass away. The majority of these illnesses are due to antibiotic-resistant pathogens, which is the reason for increased tendency toward using natural antibiotic agents. Following Previous studies that have indicated that pomegranate fruit and peel have strong antibacterial activity, in this study we have investigated antibacterial effects of different extracts of Punica granatum flowers, which are known to have tannin-rich components, against food-borne bacteria such as Staphylococcus aureus (ATCC 25923), Bacillus cereus (PTCC 1247), Escherichia coli (ATCC 25922), Shigella dysantriae (PTCC 1188), Salmonella typhi (ATCC 19430).

Methods: Antibacterial effects of different extracts of pomegranate flowers including methanol and water were evaluated using agar diffusion method. MIC values were determined using macro-dilution method according to CLSI. Both of the extracts revealed relatively high antibacterial activity.

Results: The MIC values of methanol extract against S. aureus, B. cereus, E. coli, S. dysantriae, S. typhi, were 0.781, 0.781, 12.5, 1.56, 6.25 mg/ml respectively. The MIC values of aqueous extract against S. aureus, B. cereus, E. coli, S. dysantriae, S. typhi, were 0.781, 0.781, 12.5, 1.56, 12.5 mg/ml respectively. Results obtained from this research indicated that the flower extracts have antibacterial activity against both Gram positive and Gram negative bacteria.

Conclusion: Therefore, flower extracts of Punica granatum can be used for prevention of food-borne diseases. Moreover pomegranate flowers can be applied in food industry and medicine as preservatives.

Keywords: Punica granatum, flower, food poisoning, antibacterial activity

P282: **The servey to amount of carcase Confiscate in slaughtered poultry with emphasis on microbial contamination in Khorramabad industries abattoir**

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Background and Aim: The servey to amount of carcase Confiscate in slaughtered poultry with emphasis on microbial contamination in Khorramabad industries abattoir Poultry slaughter house, is a place where the steps of: health inspection before slaughter live poultry, poultry anesthetize live, go head, bleeding, filling in boiling water bath, health inspection of carcasses after slaughter, drained viscera, washing, cutting legs, chilling and packaging. specifies observant perform by veterinary health inspectors at two particular stages (befor and after of slaughter) in addition to general controls.

Methods: During the first quarter of 1392 are slaughtered 1414642 hens in four industrial abattoir of Khorramabad. In different stages of preparation, 9174 pieces (64/0%) of them confiscate by different reasons.

Results: There are 4178 pieces (% 45/6) of this confiscate by microbial contamination such as: septicemia, peritonitis, CRD complex and respiratory disorder.

Conclusion: Prevent the risk of contamination and prevent of other carcasses to contaminate in various stages of preparation, must important for our society that to do by veterinary personnel in monitoring the health of livestock and poultry abattoirs.

Keywords: Slaughterhouse, health inspections, microbial contamination, poultry,

P283: **Tea microbiological specification**

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Background and Aim: Tea in different forms was used every day in the entire world . This type of foods need short time to heat before consumption. Usually they are contaminated with some pathogen bacteria during not suitable preparation. Due to this fact and more probability to microbial contamination, this study was accomplished to control microbial quality of this type of food

Methods: this study was fulfilled based on the national standard (NO. 2946-9263-2198-10899-2) for detection of E.coli, Coli form, Enterocci and Yeast and Molds in 117 samples.

Results: Findings of the survey showed 2 (1.13%) of the total samples were contaminated with Yeast and Molds and weren't appropriate to use.

Conclusion: Results indicated that the method of production of Tea have not good quality, and since various reports claim of inappropriate health quality of these products, more supervisions and more serious of health authorities are required to production to improve the safety of that.

Keywords: Tea, Microbiology, control

P284: **Effect of liquid smoke and smoking with Noaeamucronata on Staphylococcus aureus in culture media and Doogh**

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Background and Aim: Smoked Doogh has been produced in Kurdistan since many years ago and Noaeamucronata plant has been used for smoking of this product.Today's the most common method of smoking is using the liquid smoke.The liquid smoke offer some advantages; it is applied easily and its concentration can be controlled. It can be analyzed and, if necessary, purified to remove undesirable particles

Methods: Mueller Hinton agar was used for Kirby-Bauer test to assess the susceptibility of Staphylococcus aureus to different concentrations of liquid smoke. In order to investigate the viability of Staphylococcus aureus in Doogh containing 0.1 and 0.2% of liquid smoke and also the products that smoked with Noaeamucronatathe Baird-Parker agar, mannitolsalt agar and coagulase and catalase supplemental test was used.

Results: The zones of inhibition of bacterial growth (clear rings) around the antimicrobial disks at different concentrations of liquid smoke (0.1,0.2,0.3,0.4,0.5,0.6,0.7,0.8,0.9, 1, 2, 3, 4, 5, 100 %) were 6, 6, 6, 6, 6, 7, 7, 8, 9, 10.5, 12, 13, 13.5, 14 and 30 millimeters, respectively, that indicates the susceptibility of the organism to the liquid smoke. Also after two weeks in Doogh containing 0.2% liquid smoke and after 3 weeks in products that smoked with Noaeamucronata and those with 0.1% liquid smoke the growth of Staphylococcus aureus was not seen.In control samples the growth inhibition was observed after 4 weeks storage of product.

Conclusion: The results showed that the concentration of0.2% liquid smoke was more effective and the effect of 0.1% of liquidsmoke is similar to traditional smoking.

Keywords: Liquid smoke,Noaeamucronata, Staphylococcusaureus,Doogh

P285: **Assessment of microbial contamination in cooked foods**

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Background and Aim: Today, food borne disease is one of the major health problems in many countries and in industrialized countries occur so frequently. Food born disease in the United States after lung and respiratory diseases are of secondary importance. In Iran, we do not have data on the amount and type of infections and food born disease, but no doubt, due to the poor conditions in food preparation, the prevalence of food born disease in our country is very high. Accidental sampling and bacteriological analysis of the most important methods for bacteriological contamination of the food the aim of this study was to evaluate the bacteriological quality of cooked food.

Methods: This study was performed on 320 samples sent to the laboratory of control drug and food Tehran University of medical science. The samples were analyzed for Total Bacterial Count, Coliform bacteria, Mould count, presence Escherichia coli, Staphylococcus aureus and Salmonella spp. using standard methods.

Results: 17% (55/320) of the samples were not acceptable microbial quality. The highest contamination rate was related to the total count of bacteria 85% (47/55) and coliform 40% (22/55) and the lowest prevalence of contamination due to Mould count 9%(5/55), Escherichia coli 9%(5/55) and Staphylococcus aureus 5% (3/55) but no salmonella spp. contamination was observed in tested samples.

Conclusion: In Some foods were studied, bacterial contamination excessive than the standard. Microorganisms cause food-borne diseases are sensitive to heat therefore if food is not enough heated can contaminate consumer. Thus, further studies to determine the sources of contamination at different stages of processing and cooking and personal hygiene is necessary. The results of this study can help health officials to reduce bacterial contamination of the cooked foods.

Keywords: Microbial contamination, cooked foods, food borne disease

P286: **Preparation and antibacterial activity of chitosan nanoparticles on food pathogenic bacteria.**

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Background and Aim: The purpose of this study was to evaluate the antibacterial activity of chitosan nanoparticles against food pathogenic bacteria. Chitosan has attracted considerable interest because of their unique combination of properties, such as biocompatibility, biodegradability, metal complexation and antibacterial activity. Therefore, chitosan has a variety of current and potential applications in various fields, for example food science.The antibacterial activity of chitosan has been widely explored

Methods: Chitosan nanoparticles were prepared based on the ionic gelation of chitosan with tripolyphosphate anions.The size of the nanoparticles were determined by zeta sizer analysis. the antibacterial activity of chitosan nanoparticles against S. aureus and Listeria monocytogenes was evaluated by calculation of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC).

Results: Result show that chitosan nanoparticles had a mean diameter of 273 nm with anarrow size distribution and these could inhibit the growth of bacteria tested. Their MIC and MBC values were 3.5 µg/Ml for both of food pathogenic bacteria.

Conclusion:: Since the presence pathogenic and putrefactive food bacteria is important, finding methods to control them is necessary. Chitosan was considerabled in antimicrobial food packaging because of biodegradability, and antibacterial activity properties. Positively charged chitosan can bind to bacterial cell surface which is negatively charged and disrupt the normal functions of the membrane, e.g. by promoting the leakage of intracellular components or by inhibiting the transport of nutrients into cells.

Keywords: Chitosan nanoparticles, Antimicrobial effect, Food pathogenic bacteria

P287: **The Effect of Storage Temperature on Microbial and Sensory Properties of Traditional Lighvan Sheep Yoghurt**

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Background and Aim: Traditional dairy products have remained popular in some regions of Iran. These fermented products are preferred to industrial dairy products in Iran due to their more desirable organoleptic properties. Lighvan tin-packaged yoghurt is one of the traditional fermented products which is originated in Azerbaijan region in Iran and produced semi-industrially from fresh sheep milk using local probiotic strains. Due to having pleasant aroma, dominant microbial flora, probiotic properties and longer shelf life (3-6 months), this product has attracted the attention of dairy industry. However, yoghurt has a high tendency to chemical corruption (Proteolysis, Lipolysis) and microbial spoilage, especially at the high temperatures. This corruption causes aroma, flavor, textural and appearance defects of the product and reduces its customer friendliness. In this study, we investigate the effect of temperature on these phenomena to find an optimal range of storage temperature.

Methods: In this study, yogurt samples, after production in three different temperatures (4, 8 and -18°C), were stored for 60 days. Starter bacteria were cultured in MRS-agar and M17-agar, coliform bacteria in VRBA and yeast and mold in YGC and counted every 15 days. Samples were evaluated for their sensory characteristics (taste, aroma, texture) and overall acceptability on a 5-point hedonic scale (5 excellent, 1 unacceptable) by a panel of 13 judges selected according to their performance in a general sensory aptitude test. All sensory analyses were carried out in triplicate.

Results: The amounts of lactobacillus bulgaricus and streptococcus thermophiles on the first day of storage were 183.66x107 CFU/g and 280.66x107 CFU/g respectively. The yeast and mold were measured 0.00015x107 CFU/g on the same day. We did not observe any coliform growth in the yogurt samples. There was also no statistically significant difference ($P<0.05$) between treats in 4 and 8 °C in terms of taste. However, we found statistically significant difference ($P<0.05$) between the taste in both of these treats and the frozen treat.

Conclusion: Due to a decrease in pH level, the growth of starter bacteria in 4 and 8 °C treats decreased towards the end of the storage period. The growth of yeast and mold, on the other hand, was increasing until the day 30 and decreased afterwards towards the end of the storage period for the same reason. As Lighvan sheep yogurt has a high total solid content and since it is produced semi-industrially and with a traditional method, the probability of contamination by molds is high, especially in the packaging stage. This is confirmed by the results of our variance analysis. In addition, in the frozen sample, the amount of starter bacteria decreased statistically significantly and the growth of yeast mold stopped completely after 15 days due to sudden temperature drop. This is possibly due to the fact that the speed of chemical reaction and microorganism growth is affected by the storage temperature. Moreover, no coliform was found in the samples as the milk was heated before the process. Finally, protein denaturation due to cold shock degraded the sensory properties.

Keywords: Lighvan, traditional, yoghurt, temperature, shelf life, microbial ,properties, sensory

P288: **Evaluation antagonistic effect of lactobacillus isolated from the local dairy on some food pathogen**

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Background and Aim: Nowadays, the species of Lactobacillus which has known as probiotic are vanguard in medicine and industry. They can improve immune system. Also, some strains of Lactobacillus spp. may possess potential therapeutic properties including anti-inflammatory and anti-cancer activities. Evaluation of antagonistic activity of Lactobacillus spp. is one of the interesting researches. This activity is known to have beneficial effects against infection bacteria such as gastrointestinal pathogens especially drug resistant.The aim of this study is assessment of antagonistic effect of isolationed Lactobacillus spp. from Kerman local dairy against some bacteria such as Escherichia coli (E.coli), Staphylococcus aureus (S.aureus) and Listeria monocytogenes (L.monocytogenes) which isolated from clinical samples.

Methods: Samples were collected randomly from Kerman local dairy (including row milk, yoghurt and cheese). They were inoculated on MRS agar plate and incubated in 37ºC and microaerophillic condition. Then, the colonies were subjected to catalase test and gram staining procedure. Gram positive rod shape bacteria , non spore forming, non motile, catalas negative were identified as Lactobacillus and stored with 20% glycerol in -20 ºC. Modified double layer method was used to evaluate the antagonistic activity. 30 µl of overnight lactobacillus suspension was placed into 0.6mm well that embedded on MRS agar .After 24 h incubation overlied with melted BHI| agar. An overnight culture of pathogen strains (108 cfu ml-1) were inoculated by streaking the swab over the entire BHI surface. The plates were incubated for 24 h at 37°C and inhibition of growth was determined by measurement clear zone surrounding each well.

Results: The pattern of inhibition exerted by the Lactobacillus spp. From various sources on the pathogens was different. The maximum size of the inhibition zone was 44mm that related to lactobasillus isolated from milk against S.aureus. Average of inhibition zone of lactobacilluses isolated from different source(mili meter): L.monocytogenes S.aureus E.coli source 20 25 19 Raw milk 22 23 20 Yoghurt 17 19 17 Cheese

Conclusion: In general E.coli clinical strain was more sensitive than the other two strains and S.ureus was the most resistant genus. Isolated lactobacilluss from yogurt had the highest inhibitory effect on whole strains. Some of this lactobacillus spp. had significant antimicrobial activity and according to these results, next step for further study might be molecular identification of strains that had maximum inhibitory effect on pathogens.

Keywords: Lactobacillus spp, Antagonistic activity, Probiotic, Kerman dairies.

P289: **Survival of Commercial Probiotic Bacteria Lactobacillus casei in Industrial & Traditional Iranian (Nehre) Butters**

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Background and Aim: Probiotics are live microorganisms which when administered in adequate amounts confer a health benefit on the host. Among the different foods act as a probiotic carrier, dairy products have special importance. Butter is a dairy product made industrially by churning sweet or fermented cream, but another type of butter produced in west and northwest province of Iran as traditionally through churning of soured milk named “Nehre”. Due to lack of findings about use of butter as a probiotic carrier, in this study efficiency of industrial and traditional Nehre butters production with strains of Lactobacillus casei was investigated.

Methods: Surviving of probiotic strains and sensory evaluation of butters were evaluated at 90 days. Probiotic cultures of Lb. casei LAFTI L26 were obtained from DSM Food Specialties. Pasteurized cream(with 33% fat) and milk(with 3.8% fat) were used to manufacture industrial and traditional butters respectively and probiotic cultures applied as direct inoculation to ferment cream and milk which converted to butter in last stage.Viable cells were enumerated by plating diluted samples (peptonized water) on solid MRS agar. Cultures were incubated for 72 h in anaerobic jars at 37 °C to determine Lactobacillus casei population.

Results: The results showed that the numbers of Lactobacillus casei in the butter samples were 8.41 log cfu/gr at the first day of production. These amounts reached to 6.13 log cfu/gr and 6.02 log cfu/gr in industrial and traditional butters after 90 days of storage time respectively.

Conclusion: The numbers of live probiotic cells were higher than those recommended for beneficial effect at the end of 90th day. Results of sensory evaluation by panelists showed that butters with Lactobacillus casei had no distinct difference in organoleptic properties. Therefore probiotic Lactobacillus casei can be used successfully in industrial & traditional butter without adversely affect on the butter quality during storage.

Keywords: butter, Lactobacillus casei, Nehre, probiotic strains

P290: **Molecular Detection of Staphylococcus aureus enterotoxin (SEC) producing strains**

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Background and Aim: Enterotoxin producing Staphylococcus aureus is one of the most common agents of bacterial food poisoning outbreaks. Milk and its products have suitable substrates for S.aureus growth and enterotoxin production. This organism is able to grow in a wide range of temperatures, pH and sodium chloride concentrations, therefore food poisoning because of S.aureus contamination is potential risk of the consumption of dairy products. Although the pasteurization kills the bacterium but thermo stable S. aureus enterotoxins (SEs) generally retain their biological activity. These toxins are produced by 30 to 50 percent of S. aureus strains, therefore are of high importance in public health and food industry .There are several types of SEs (A, B, C, D, E, G, H, I, J, K, L, M, N, O, P, Q and R) and the corresponding gene that have been described to date. Types A,B,C,D and E of SEs are especially responsible for food borne poisoning. Considering the above mentioned facts, the aim of present study was to determine the frequency of S.aureus contamination and presence of sec gene encoding the Staphylococcal enterotoxin (SEC) in S.aureus isolates from raw and pasteurized milk and ice cream samples by using PCR technique.

Methods: In this study, a total of 100 samples were collected from September to December 2012 in Karaj city. 50 samples of milk and 50 samples of ice cream were purchased from different markets. Mannitol salt agar medium was used for Detection and isolation of S.aureus from the samples. DNA of the samples were extracted using DNG TM plus kit, (Cinaclon Co. Iran) according to the manufacturer's recommended procedure. Standard strains of S.aureus were used as positive controls.PCR was optimized to amplify S.aureus specific thermonuclease encoding gene (nuc) and second round of PCR was optimized to amplify 283 bp segment of sec gene performed on positive samples. PCR products were evaluated by agarose gel electrophoresis

Results: According to the results of the culture based methods 34 out of 100 samples were detected as mannitol fermenter S.aureus .Our PCR results demonstrated that S.aureus frequency was 57% in the total samples, and 10.52% of (6 out of 57) positive samples contained sec gene.

Conclusion: According to the social context and geographical situation of Karaj city and also the frequency and variety of traditional and industrial centers of dairy supply , it seems that PCR method can be useful for S.aureus detection as a rapid , highly specific, and sensitive alternative to microbiological method with the potential for providing of improved food-processing hygiene control.

Keywords: Staphylococcus aureus, dairy products, enterotoxin C, PCR, food poisoning

P291: **Microbial Contamination in Traditional Icecream and Effective Factors,Sari 2012**

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Background and Aim: Background and purpose: Ice cream is a frozen dairy product which is contaminated easily due to its nutrient invironment and could endanger people's health through food poisoning.The aim of this study was to determine the microbial contamination in traditional ice cream and factors affecting it.

Methods: Materials and Methods: This study was carried out in summer of 2012 using 50 samples of ice cream,row materials ,scoops,containers,and staff's hands.The samples were transported in sterile conditions to the Food Conrol Laboratory.In less than one hours, the samples were tested using both standard and direct culture.After 24-48 hours incubation,biochemical tests were performed to identify pathogen bacteria according to the text books.

Results: Results: The results showed that 84% of traditional ice cream were contaminated.The contaminations included Staph aureus(28%),Escherichia coli(52%),Bacillus cereus(4%),Listeria monocytogenes(2%),Enterococcus spp(14%),enumeration of enterobacteriaceae(70%),total count of microorganisms(74%).There was also a significant correlation between contamination in ice cream and row materials,scoops,containers,and staff's hands.

Conclusion: Conclusion: The main sources of contaminants for traditional ice cream were row materials such as milk,sugar,vanilla,utensils and staff's hands.High levels of contamination in traditional ice cream and the risks of consumption indicates a serious need for greater control over production units and encouraging the public to use pasteurized ise cream through mass media.

Keywords: Key words: Ice cream, Microbial contamination,pathogen bacteria.

P292: **Survey Production of Lactic Acid by lactic acid bacteria isolated from traditional cheese of the Khorramabad city**

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Background and Aim: The characteristics of acid production, technical features important considered for the dairy industry. Fall production of lactic acid, suggested all isolates that their ability to produce lactic acid after 6 h of incubation up 2/5 mg/ml (acidity> 2.5 mg / ml) can be used as the starter culture in cheese manufacturing. For this purpose lactic acid production by isolated from traditional cheese of Khorramabad city were evaluated

Methods: To determine the active acidity, after identification of strains by using the method of phenotype (morphology, Gram stain test, physiological and biochemical), Acid production ability in milk is one of the most important technological characteristic of LAB. In order to determine acidifying activity, potantiometric (pH measurement) and titrimetric methods were applied. For this purpose, isolates were activated from frozen stocks in MRS broth for 24 h at 30°C and 0.1 ml overnight cultures were inoculated in 10 ml of sterile UHT skim milk broths. Duplicate inoculations were prepared. After the 3hth, 6hth ,9hth and 24 hth incubations at 30 °C, 2 ml aliquots were taken aseptically, and used for the procedures pH of samles were determined according to the potentiometric method. Onto the 2 ml of aliquots, 1-2 drops of phenolphatlein solution were added as indicator. Samples were then titrated by using standardized (F = 0,8816) 0.1 N NaOH solutions. When the first trace of pink color was observed ,titration was terminated.

Results: We isolated strains of isolation and identification of Lactobacillus plantarum, Enterococcus. faecium, Enterococcus hirae, Lactococcus lactis, respectively. After 6 and 24 h, respectively, producing lactic acid for Lactobacillus plantarum 3/38 and 5/65 mg/ ml, for E. faecium 3/38 and 5/20 mg/ml, for Enterococcus hirae 3/30 and 4/73 mg/ml and for Lactococcus lactis 3/06 and 3/60 mg /ml respectively.

Conclusion: All isolates due to high levels of lactic acid can be used as the starter culture in cheese manufacturing.

Keywords: production of lactic acid, lactic acid bacteria, cheese, Khorramabad

P293: **Enterotoxin A gene within Staphylococcus aureus isolated from raw red meat and poultry in Tehran**

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Background and Aim: Staphylococcus aureus is the most common agent of food borne illnesses. S. aureus produce enterotoxins (SEs), which are the main cause of food poisoning. Staphylococcal enterotoxin A is one of the most important causes of gastroenteritis. The aim of this study was to determine the S. aureus contamination of meat material and to detect the enterotoxin A gene within raw meat samples collected from Tehran dealers.

Methods: In this descriptive cross-sectional study, 186 samples of raw meats were taken randomly from retail butcheries and supermarkets. The samples were prepared and cultured for S. aureus. The isolates were then identified by routine bacteriological methods. The isolates were subjected to PCR (Polymerase Chain Reaction) for detection of the gene-encoding SEA.

Results: The results indicated that 29 out of 186 (15.6%) raw meat samples (raw beef 14.8%, raw lamb 15%, raw chicken 15.7% and raw turkey 16.6%) were contained S. aureus. 5 samples including 2 raw chicken, 1 raw turkey, 2 raw beef were found to be positive for SEA gene. None of lamb meat samples harboured SEA gene.

Conclusion: Although the presence of enterotoxigenic strains in food does not always necessarily mean that the toxin will be produced, our results suggest that it is of special importance to follow the presence of enterotoxigenic S. aureus strains in meat products, especially for protecting the consumers from food poisoning since pH and aw values of these kinds of products are favorable for S. aureus growth.

Keywords: S. aureus, Enterotoxin, SEA, Food poisoning, Raw meat

P294: **Evaluation Proteolytic effect of Lactic Acid Bacteria on Bovine Betalactoglobulin to reduce its allergenicity and bioactive peptide production**

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Background and Aim: Betalactoglobulin (BLG) is the most abundant protein in whey of ruminant´s milk and considered the major cause of milk allergenicity especially in infants. This protein is stable at low pH and cause resistant to gastric digestion, so protein can maintains its allergic epitopes. Lactic Acid Bacteria (LAB) are the most important group of starters in fermentation of dairy products, because of their metabolic activity on proteins. Recently afew LAB strains have been extensively studied for their proteolytic system to hydrolyze the whey proteins and produce bioactive peptides. The aim of this study is to evaluate the ability of three types of LAB to hydrolyze of BLG in order to reduce its allergenicity.

Methods: we used the fresh culture of Lactobacillus casei, Lactobacillus paracasei, Lactobacillus delbrueckii subsp bulgaricus in milk citrate agar medium and the cultures were incubated at 37°C for 48h. The collected cells were washed twice with solublization buffer and resuspended to final OD 600 of 20 in 100mM sodium phosphate buffer (pH 7.2) and were mixed with dissolved BLG. Protein hydrolysis and peptide formation were analyzed by SDS-PAGE and Tricine SDS-PAGE.

Results: Analysis of SDS-PAGE after 24-48h incubation showed that, the strains can hydrolyze bovine BLG and produce peptide bonds.

Conclusion: Lactobacillus strains are able to hydrolyze BLG and might break down its allergic portion. The strains also might produce reduced allergic peptides with various biological activity like immunomodulatory properties.

Keywords: bovine betalactoglobulin,Lactic Acid Bacteria,allergenicity,bioactive peptide

P295: **The effect of heat stress in broiler with role the Bio-SAF47 based probiotic and Bio-Mos based prebiotic: secretion of cortisol hormone**

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Background and Aim: Heat stress (HS), one of the major problems in tropical and subtropical countries, which adversely affect the production performance of poultry . Keeping this in view, The present study was designed to investigate some of the biological markers of HS in broilers as modulated by dietary supplementation of mannan-oligosaccharide (MOS) and as prebiotic and probiotic Saccharomyces cereviciae BIOSAF, either alone or in combination

Methods: One hundred 1-day-old chicks were randomly divided into five groups. From d one, the birds in the 1 - standard temperature thermoneutral zone (TN) and 2 – HS, HS-BIOMOS, HS-BIOSAF and symbiotic Group. Birds were fed either a corn-based basal diet (TN and HS Group) or the same diet supplemented with 2,1, 0.5% MOS (HS-MOS group), 0.5% BIOSAF (HS-BIOSAF group), or their combination according to the recommendations of the manufactur in . each of 5 Groups the Birds were immunized against on the first day by the Infectious bronchitis 4/91 of spray (live attenuated) were vaccinated, and on day nine of influenza - Newcastle vaccine Co. Pasouk the infusion were vaccinated at 14 days against bursal disease virus infection by vaccine Gambokal (using eye drops) (live attenuated) were vaccinated chickens on days 21, 35 and 42 with a syringe, 2 ml samples were then Serum collected at a temperature of - 20 ° C was maintained after 42 days, to determine cortisol hormone (ELISA method),

Results: we apply the results show The use of dietary supplements in group under HS compared with TN and reduced serum cortisol,and¬¬¬¬ this decrease¬¬ was statistically¬¬,significant.(P?0.05) A comparative examination except Duncan Group HS-BIOMOS TN group was not significant (p>% 074).

Conclusion: we can conclude that the use of dietary supplements BIOMOS and BIOSAF alone or as Symbiotic can have some harmful effects, reduces heat stress in broilers

Keywords:: heat stress, probiotic Bio-SAF 47, prebiotic Bio-MOS, cortisol hormone

P296: **Production of natural lactons as a bioflavor by yeasts**

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Background and Aim: Flavors play an important role in food industries. In dairy industry, flavor is a unique attribute which determines its acceptability. Also flavor can be described as the combined perception of taste, smell and mouth feel, Moreover flavors increase consumers response towards "Natural" ingredients. However, chemical synthesis results many problems, such as environmentally unfriendly production processes and undesirable racemic mixture compounds. Hence, biotechnology of flavors shows their necessity. The aim of this study was is to research the abilities of some yeasts such as Saccharomyces cerevisiae and Yarrowia lipolitica to produce deca-lactone and decanolide as a stabilized natural flavor for using in dairy products

Methods: For production of d-decalactone, The α,β-unsaturated lactone which is the main component of Massoi bark oil, can efficiently converted by Saccharomyces cerevisiae into d-decalactone in a controlled biological process. But Most of the commercial processes for the production of decanolide are based on the Ricinoleic Acids)Natural Hydroxyl Fatty Acids(. Ricinoleic acid, the main fatty acid of castor oil, or esters thereof as substrates and fatty acid degrading yeasts (e.g. Yarrowia lipolitica) or a higher fungi as biocatalysts.

Results: The process for which high product concentrations have been reported by Haarmen and Reimer are based on strains ofYarrowia lipolytica, a yeast which is particularly well adapted to hydrophobic environments and which was patented for 4-decanolide production for the first time. Ricinoleic acid, is degraded by four cycles of β-oxidation and one double-bond hydrogenation into 4-hydroxydecanoic acid, which lactonises at lower pH to 4-decanolide, resulting in the same R configuration of the lactone as is found in peaches and other fruits . By controlling the process, they could obtain up to 11 g/L 4-decanolide in 55 h with raw castor oil as the substrate by Yarrowia lipolytica. Subsequently producing of this bioflavor has resulted US $ 300 per kilogram. Also octanolide naturally found in meat, cheese, can be produced as a by-product besides decanolide when a mixture of 11-hydroxypalmitic acid and 3,11-dihydroxymyristic acid from Jalap resin is converted by Saccharomyces cerevisiae.

Conclusion: Biotechnological processes can be used as an alternative to natural compounds, it is essential to know that the use of bioflavors can enhance the safety and quality of foods. Hence work is needed for future developments.

Keywords: Bioflavor, Yeasts, Natural lactons, Yarrowia lipolytica, Saccharomyces cerevisiae

P297: **Influence of yeast strain on Shiraz vinegar quality indicators**

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Background and Aim: vinegar styles are defined by complex and highly diverse chemical compositions. Evidence suggests that some of this complexity is determined by the choice of yeast strain used in fermentation. There are hundreds of different commercially available vinegar yeast strains that, potentially, provide a means by which winemakers can tailor their vinegar for different consumer market segments.n this study we evaluated the impacts of fermenting Shiraz must with different yeast strains, with a focus on chemical composition and tannin content of the finished vinegar.

Methods: Principal Component Analysis (PCA) of the vinegar indicated that choice of yeast strain had a strong influence on a number of vinegar compositional parameters, including tannin.

Results: In three fermentation experiments, across two vintages and using different winemaking protocols, a compelling case for yeast strain 'signature' was evident.

Conclusion: The results demonstrate that there is an opportunity to use commercial vinegar yeast diversity to modulate vinegar composition and, by implication, the style of finished vinegar.

Keywords: Yeast strains; Multivariate data analysis; Saccharomyces cerevisiae; Saccharomyces bayanus

P298: **Aflatoxin-producing Aspergillus species from Rafsanjan.**

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Background and Aim: Aflatoxins are naturally occurring mycotoxins that are produced by many species of Aspergillus, a fungus, the most notable ones being Aspergillus flavus and Aspergillus parasiticus. Their name is derived from the early work that discovered Aspergillus Flavus toxins. Aflatoxins are toxic and among the most carcinogenic substances known.Aflatoxin-producing members of Aspergillus are common and widespread in nature. They can colonize and contaminate grain before harvest or during storage. Host crops are particularly susceptible to infection by Aspergillus following prolonged exposure to a high-humidity environment, or damage from stressful conditions such as drought, a condition that lowers the barrier to entry. The native habitat of Aspergillus is in soil, decaying vegetation, hay, and grains undergoing microbiological deterioration, and it invades all types of organic substrates whenever conditions are favorable for its growth. Favorable conditions include high moisture content (at least 7%) and high temperature.

Methods: Aflatoxin-producing Aspergillus species were isolated from soil samples from ten different regions within Thailand. Aspergillus flavus was present in all of the soil samples and then Measurement of aflatoxin production on defined media and Sequencing and phylogenetic analyses.

Results: Unlike previous studies, we found no A. parasiticus or A. flavus capable of both B- and G-type aflatoxin production in any of the samples. A. pseudotamarii, which had not been previously reported from Thailand, was found in four soil samples. In two of the samples A. nomius was determined to be the most abundant aflatoxin-producing species. Based on sequence alignments for three DNA regions (Taka-amylase A (taa), the rRNA internal transcribed spacer (ITS), and the intergenic region for the aflatoxin biosynthesis genes aflJ and aflR) the A. nomius isolates separated into three well-supported clades. Isolates from one of the A. nomius clades had morphological properties similar to those found for S-type isolates capable of B and G aflatoxin production and could easily be mistaken for these isolates.

Conclusion: Our results suggest that such unusual A. nomius isolates could be a previously unrecognized agent for aflatoxin contamination in Rafsanjan.

Keywords: Aflatoxin; Phylogenetics; Aspergillus nomius; Flavus

P299: **Antibiotic resistance profile of Vibrio alginolyticus isolated from a freshwater fish (Scomberoides commersonnianus)**

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Background and Aim: Extensive use and misuse of antibiotics in medication, veterinary, agriculture and aquaculture have caused the wide spread increased nature of antibiotic resistant bacteria. In addition, bacteria, that are pathogenic to humans may occur naturally in farmed fish or aquatic environments and make their way to humans with spread of resistance genes, leading to health problems. Thus, foods contaminated with antibiotic resistant bacteria is a threat to public health as the antibiotic resistant determinants may be transferred to other bacteria of clinical significance. Among this, V. alginolyticus has categorized as one of the seven Vibrio fish pathogens and are frequently isolated from outbreaks and mortalities in many fish species. Thus, with regarding to the importance of this pathogen and probability of its antibiotic resistance, the goal of this research was determination of antibiotic resistance profile in order to control of fish's disease rising from this bacteria.

Methods: Following the enrichment of fish samples, identification of Vibrio alginolyticus isolates performed by biochemical and microbiological tests. Antibiotic resistance patterns of these isolates was determined by the disc diffusion method with the use of Mueller-Hinton agar, according to the Kirby-Bauer method. Disks containing the following antibacterial agents were placed on the plate and incubated overnight: Ciprofloxacin (Cf-5µg), Trimethoprim (Tr-5 µg), Tetracycline (T-30 µg), Erythromycin (E-15 µg). After incubation, the diameter of the zone of inhibition was measured and compared with zone diameter interpretative chart to determine the sensitivity of the isolates to the antibiotics. The results interpreted as sensitive, Intermediate and resistant according to CLSI recommendations.

Results: among the 50 samples which isolated at the primary isolation, 5 (10%) isolates was identified as V. alginolyticus. This isolates were subjected to furthur tests including antibiotic resistance pattern identifiction. It was revealed that all isolates were sensitive to tetracycline (< 14mm) and trimethoprim (> 16mm); 3 isolates showed intermediate pattern to ciprofloxacine (16-20mm) and 2 isolates have sensitivity toward this antibiotic (> 21mm). 4 isolates were resistance to erythromycin (< 13mm) and only one isolate showed intermediate pattern to this antibiotic (14-22mm).

Conclusion: V. alginolyticus is known as an opportunistic pathogen in human as well as marine animals. Special attention to V. alginolyticus zoonotic infections should be taken urgently in consideration because its transmission is possible via infected fish and sea water. Since, in this research revealed that all isolates were sensitive to tetracycline, we can say this antibiotic could exploited to control of fish disease which rise from V. alginolyticus.

Keywords: antibiotic resistance, Vibrio alginolyticus, freshwater fish

P300: **Improvement and optimization of multiplex PCR amplification of serovar-specific genomic regions (SSGRs) for direct detection and serotyping of Salmonella Typhimurium in milk**

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Background and Aim: Contamination of Milk with Salmonella is a major risk factor for human health and multiple outbreaks of salmonellosis by milk contamination have been reported. To decrease the time and labor requirements of conventional detection methods, rapid and sensitive methods mainly based on PCR amplification are preferred. Direct and rapid serotyping can be a useful tool for epidemiological monitoring and better control of the outbreaks. The aim of the present study was improvement and optimization of multiplex PCR amplification of serovar-specific genomic regions for the direct detection and serotyping of Salmonella Typhimurium in milk.

Methods: Samples of previously sterilized milk were inoculated with 10 to 105 CFU/mL of Salmonella Typhimurium LT2 and tested for multiplex PCR of 4 sequences including invA gene as the marker of Salmonella and 3 SSGRs specific for Salmonella Typhimurium. Direct plate counting and parallel PCR with filter-purified extracted DNA were simultaneously performed to validate the results. After several tests for the improvement and optimization of reactions, the lowest detection limit of the method for milk samples was determined.

Results: The best results were found by direct PCR with 1 microlitre of supernatant of samples after boiling in 100°C for 10 minutes and 10,000 RPM centrifugation. The best PCR condition was found with initial denaturation of 95°C for 3 minutes followed by 30 cycles containing Denaturation (94°C, 45 sec), annealing (60°C, 30 sec), extension (72°C, 45 sec), and final extention of 72°C, 7 min. Although the detection limit was 10 CFU/mL, the sensitivity decreased in this concentration especially for larger PCR products.

Conclusion: Results of this study in conjunction with control evaluations proved that this method can be used routinely as a sensitive and rapid method with overall 3 hours time requirement for the detection of Salmonella Typhimurium in milk samples.

Keywords: Multiplex PCR, Milk, Salmonella Typhimurium, SSGRs

P301: **Inhibitory activity of Lactobacillus rhamnosus and Lactobacillus delbrueckii subsp. lactis supernatants against Pseudomonas aeruginosa**

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Background and Aim: To minimize antibiotic resistance, antibiotic consumption should be limited by providing an alternative method. This experimental study aimed to investigate the inhibitory activity of two probiotic bacteria against several strains of Pseudomonas aeruginosa.

Methods: In this study supernatants obtained from bacteria Lactobacillus rhamnosus and Lactobacillus delbrueckii subsp. lactis and their inhibitory activities investigated against 45 isolates of Pseudomonas aeruginosa using two methods of disc plate and well after 24 hours of incubation. Zone of growth inhibition around the wells, and the discs were measured. Results were evaluated with the software spss18 and antagonistic probiotic bacteria were compared.

Results: Inhibitory effects of the probiotic bacteria was observed on the growth of most isolates of Pseudomonas aeruginosa. Maximum inhibitory effect on well method belonged to Lactobacillus rhamnosus strain by mean inhibition zone 10/65 ± 1/48 mm and a minimal inhibitory effect on well method belonged to Lactobacillus delbrueckii subsp. lactis by mean inhibition zone 8/43 ± 1/6 mm. Maximum inhibitory effect on disc plate method belonged to Lactobacillus delbrueckii subsp. lactis by mean inhibition zone 6/75 ± 0/56 mm and a minimal inhibitory effect on disc plate method belonged to Lactobacillus rhamnosus by mean inhibition zone 6/22 ± 0/4 mm.

Conclusion: Supernatant from these probiotic bacteria shows remarkable activity against Pseudomonas aeruginosa isolates from patients. These results suggest that the use of these bacteria as a potential candidate for the prevention of nosocomial infections. Furthermore, this research shows that the method of well disc is superior to well method for measuring antibacterial activity.

Keywords: Inhibitory activity, Lactobacillus rhamnosus, Lactobacillus delbrueckii subsp. lactis, Pseudomonas aeruginosa

P302: **Microbial evaluation of fresh, minimally processed vegetables, and Bagged sprouts from chain supermarkets**

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Background and Aim: Increases in the worldwide consumption of ready-to-eat (RTE) produce have resulted in increases in food-borne illness associated with these products. Previously there was no available information on the levels of indicator bacteria and the microbial quality in Minimally Process Vegetables (hence forth MPV) and sprouts. The aim of this study was to characterise the microbiological quality of selected common ready to eat vegetables and sprouts commercially sold in Iran in order to provide insight into any potential health hazards associated with consumption of these commodities.

Methods: A total of 116 samples of fresh cut vegetables, ready-to-eat salads, mung bean and wheat sprouts, collected from major supermarkets and local markets, were tested for aerobic mesophilic counts and enumeration of yeasts and moulds, enumeration of coliforms and detection of Escherichia coli O157: H7 and Salmonella spp

Results: Aerobic mesophilic bacteria in fresh cut vegetables and fresh mung bean sprouts with the population of 5.3 and 8.5 log CFU/g were the lowest and highest loads, respectively. E. coli O157: H7 was resulted to be absent in all samples, but E. coli was detected in 21 samples (18.1%) and salmonella spp. was found in one mung bean (3.1%) and one ready-to-eat salads sample (5%). Yeasts were the predominant organisms and found in 100% of the samples. In mung sprouts (Geotrichum, Fusarium and Penicillium spp.) and ready-to eat salads (Cladosporium and Penicillium spp.) constituted the most prevalent molds, which may predispose mycotoxins production.

Conclusion: According to the results, Although no E. coli O157: H7 was detected in the Minimally Process Vegetables and sprouts analysed in this study, high bacterial and fungal counts, especially in bean sprouts and fresh-cut salad, imply that effective control measures should be implemented to improve the microbiological quality of fresh produce sold in Tehran, Iran.

Keywords: Microbial safety, Fungi contamination, Sprout, Foodborne pathogen, Mold.

P303: **Effect of Ultra-Low Intensity Direct Current (U-LIDC) on Growth of Escherichia coli**

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Background and Aim: Eradicating effect of electrical current has been shown in several microorganisms. This methodology has been primarily employed to destroy microorganisms in waste water, apiculate yeast in wine making, sterilization of water, pasteurization of milk, etc. However, there are limited information available on the excitatory effects of low intensity electric current on eukaryotic cells (wound healing), bacteria and yeasts. The lowest current reported in the literature is 5 µA/cm2. In the current study, for the first time the effects of ultra-low intensity direct current (U-LIDC) on the possibility of promoting growth of Escherichia coli were studied.

Methods: Rode electrode made of stainless steel 316L (used in biological and food industries), Escherichia coli (pathogenic strain isolated in the laboratory), and nutrient broth (Merck KGaA, Darmstadt, Germany) were used in the study. A 100 mL glass cell with cup was attached to a DC power supply set to deliver a constant voltage (400±0.5 mV adjusted by precise multi-meter Sanwa, PC5000, Japan) for 18 hours (in 11 time points sampling). Two replicated cells were used with arrangement of negative control (no electrical connection, no bacteria), positive control (no electrical connection, inoculated), positive plus negative, positive plus ground, negative plus ground, positive alone, negative alone and ground alone. Changes in absorbance (Spectrophotometer: PG Instrument, T80+, OD 625 nm) were recorded for growth of bacterial population.

Results: Mean of absorbance during 18 hours for each cell type were recorded as 0.021, 0.197, 0.255, 0.269, 0.275, 0.263, 0.279, and 0.273 respectively. Compared with positive control, there were statistically significant ($p < 0.05$) differences in the mean values at all groups except group ground alone. Compared between two replicates, there was no statistically significant difference in the mean values of both last harvests.

Conclusion: Our findings show that the U-LIDC created by potential difference of 400 mV enhanced growth of Escherichia coli significantly. Based on these results, we recommend employing U-LIDC in biological fermenters as an alternative methodology for achieving greater microorganism production.

Keywords: Ultra-low direct current Escherichia coli

P304: **Primary characterization of an antibacterial substance from Prosopis juliflora**

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Background and Aim: Plants are hopeful sources for new antibacterial agents. Prosopis juliflora, which belongs to the family of Fabaceae, is an endemic plant flora of some different parts of the world and also can be find in south of Iran. This plant has been known as anti infective in traditional medicine of Iran.

Methods: . In this study, organic solvents (Methanol, n-Hexan and Ethyl acetate) were used for extraction of plant materials from leaves of the plants by standard procedure and then antibacterial activities of the extracts were evaluated. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the plant materials were determined against 6 bacterial standard strains and 1 yeast, by broth micro-dilution method as recommended by CLSI (Clinical laboratory Standard Institute) with some modifications. Chloramphenicol was used as standard antibiotic. Microbial strains contained Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa (3 different strains) and Acinetobacter baumanii. A flash chromatography was conducted in parallel to a bioautography method based on high performance thin layer chromatography.

Results: The lowest recorded MICs were 0.075 and 0.125 mg/ml for n-Hexan extract of leaves against E. coli and S. aureus strains respectively. However the highest MICs values were 16 mg/ml, when the same extract was tested against C. albicansP. aeruginosa. It was surprising that Ethyl acetate extract of leaves could inhibit 3 tested strains of P. aeruginosa in concentrations ranging from 0.15 to 0.75 mg/ml.

Conclusion: According to the results of this study, Prosopis juliflora could be considered as a hopeful source against some important human pathogenic bacteria which some of them could be inhibited by 0.016 and 0.04 mg/ml of Chloramphenicol. Determination of the effective antibacterial substances of the plant leaves is underway in our Institute using HPTLC and reverse phase-HPLC.

Keywords: Prosopis juliflora; Antibacterial activity; MIC; MBC; HPTLC, HPLC

P305: **Evaluation of some Iranian medicinal plants and their constituents for their anti Helicobacter pylori activities**

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Background and Aim: Helicobacter pylori is a Gram negative bacterium which is associated with peptic ulcer, chronic gastritis and other stomach diseases. According to high prevalence of infection by this microorganism, any food plants with H. pylori inhibitory effects could be helpful in prevention and even in erasing of the infection.

Methods: Anti H. pylori activities of essential oils derived from Cuminum cyminum, Mentha longifolia, Rosmarinus officinalis and Zingiber officinalis plants and some purified coumarins of Ferula spp. were assessed by in vitro antibacterial activity using standard disk diffusion method and by determination of minimum inhibitory concentration in standard broth dilution method. 2 clinical strains which were isolated from biopsies and one standard strain were included in the study. Clarithromycin was used as standard antibiotic.

Results: Our preliminary results showed that Cuminum cyminun with the inhibition zone of 16 mm had the best antibacterial activity followed by Z. officinalis (12.5 mm), M. longifolia (12.25), R. officinalis (10.5 mm). Primary results with tested coumarins could indicate the effectiveness of this compounds in vitro, with MIC values ranging from 16 to 128 microgram/ml

Conclusion: Our study could indicate anti H. pylori activities of some medicinal plants. In this regards disesquiterpene and sesquiterpene coumarines of Ferula spp. could be considered the most effective samples which were assessed in the present work. This results could be hopeful for considering medicinal plants and their constituents as effective anti Helicobacter pylori agents

Keywords: Helicobacter pylori, Essential oils, Medicinal plants

P306: **Dissemination of extended-spectrum beta lactamases (ESBLs) genes among clinical isolates of uropathogenic Escherichia coli in children**

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Background and Aim: Urinary tract infection (UTI) is one of the most common childhood bacterial infectionsand E.coli is the major pathogen. The aim of this study was to determine the prevalence of ESBLs genes in E.coli strains isolated from UTIs.

Methods: In this study, a total of 120 isolates of E.colifrom urinary tract infections of the children, which were collected at BESAT hospital in Hamadan, west of Iran, from October 2010 to October 2011, included. The isolates diagnosis was done by standard biochemical methods. Antimicrobial susceptibilities were determined by disk diffusion method, ESBLs- producing was confirmed phenotypically using the double-disk synergy (DDS) test.The presence and identity of ESBLs genes were determined by polymerase chain reaction (PCR).

Results: The highest sensitivity was seen respectively to Imipenem (96.7%),Amikacin (92.5%), Gentamicin (70.8%) and Ciprofloxacin (79.2%); in contrast the highest rate of resistance was seen for Cotrimoxazole (77%), Cefotaxime (36%).

Conclusion: CTX-Mwas the most prevalent ESBL genotype in uropathognic E. Coli (UPEC) isolated from UTI. It is recommended that in order to avoid treatment failures, use phenotypic and molecular methods for experiments related to diagnose these enzymes genes.

Keywords: Escherichia coli (E.coli), ESBLs, UTI, Antibiotic resistance

P307: **Design and fabrication of nanobiosensor based on lspr and it's application in Staphylococcus aureus detection**

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Background and Aim: Food and water safety depends on ability of detection their pathogens. There are several virus and bacteria are caused to food borne diseases that Staphylococcus aureus is one of the most important agents. Food analysis methods should be able to identify pathogen agents and detect of food adultration with high sensitivity and directly that LSPR nanobiosensors can be the most important alternative in this field.

Methods: In this project NSL(nanospher lithography) is used for fabricating Ag-Au nanoparticles with glass substrate . After deposition process by vacuum evaporation system, assessment of our nanoparticle array is performed by SEM and uv- Visible spectrometer.Then,the nanoparticles are functionalized with specific agents in multistep process.In this study the lspr spectra for the specific binding signals are measured using the integrated LSPR biosensor.

Results: Results of several research groups have explained that detection of chemical and microbial agents in food stuff and clinical samples by LSPR nanobiosensors can be the most promising and widely development method in future.

Conclusion: This kind of nanobiosensor can be identify Staphylococcus aureus and other bacteria directly and specifically.

Keywords: nanobiosensor- LSPR(localized surface Plasmon resonance) - Staphylococcus aureus

P308: **The Process of teaching and Presentation Microbiological M.Sc. Seminar Course**

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Background and Aim: The purpose of this study was offer an applicable method in order to presentation of seminar course for M.Sc. students.

Methods: A cross-sectional study was designed a questionnaire to evaluate and measuring the skills students learned in the seminar course. Two groups student consisting of five persons who participated in the implementation of this Procedure. 5 sessions of 2-hour seminar was intended to teach. The first meeting of the purposes of evaluating the students' academic level seminar and workshop topics were selected for discussion between teachers and students. Students were placed in the program for the next session. The interval of each session was at least 20 to 30 days. Finally, the post test was taken out. The sum of the scores obtained were subject to descriptive analyzes. The score was calculated for students attending the session.

Results: The Results of this study was to design a valid questionnaire for evaluation skills and activities for students. The questionnaire consisted of 5 core issues and had a several minor issues with the Measurement index. Each minor issue was assigned a score from 0 to 200. The total score of 4800 questionnaires were completed. The most important evaluation issues were named as: Scientific survey, production of knowledge, self-reliance, competition, application, creativity and innovation, relying on domestic resources, the funds development, and native knowledge. The preliminary evaluation of students for each of the issues considered was less than 50. While, after conducting meeting five, the students were achieved score of at least 150 of the 200 from each issue. This shows the increasing capabilities of students. The final step in the evaluation of student achievement scores were in 4500.

Conclusion: Recently, university courses such as seminar have been widely addressed in academicians. In this study, the process of presenting seminars of M.Sc. students was designed. The results revealed that this method could be based on several targets, including moving the frontier of knowledge, sources of knowledge, ways of the study, preventing of plagiarism, scientific review and analysis, can improve the students' creative power. Because of the student’s pre-test scores were 50 in each issue while post test scores came out reached to 200? Therefore, it is suggested that this method could be used for all M.Sc. and Ph.D. students.

Keywords: Seminar Course, Lecture, Acquiring Skills, M.Sc. Student

P309: **Fungal infections of respiratory tract**

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Background and Aim: Fungal infections of the respiratory tract are important causes of morbidity and mortality in immunocompromised patients. Invasive fungal infections has been reported with increased frequency in parallel with an expanding population of immunocompromised patients (solid organ transplantation, Bon marrow transplantation, cancer) .The aim of this study was diagnosis fungal infections of respiratory tract.

Methods: From November 2008 to February 2011, 244 immunocompromised patients were evaluated for respiratory tract infection, having been suspected by clinician (clinical and radiological), the clinical samples (biopsy, broncho alveolar lavage and sputum) were examined for fungal infection. All samples were cultured on Sabouraud Dextrose Agar with chloramphenicol and direct microscopic examination was performed with potassium hydroxide. Fungi are identified on the basis of gross and microscopic morphologies. For yeast identification germ tube and API test were carried out.

Results: Fungal respiratory tract infections diagnosed in 34 patients (14 sinusitis and 20 pneumonitis). Etiologic agents for sinusitis were Aspergillus flavus 6 cases, Aspergillus fumugatus 3 cases, Alternaria 3 cases and Mucor 2 cases. Etiologic agents for Pneumonia were Aspergillus flavus 4 cases, Aspergillus fumigatus 3 and Candida albicans 13 cases (diagnose with individual criteria).

Conclusion: In patients with signs and symptoms of respiratory tract infection, radiology and laboratory findings are necessary to show the value of imaging

Keywords: immunocompromised patients, Aspergillus, Candida albicans

P310: **Diagnosis methods for the diagnosis of fungal endocarditis**

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Background and Aim: Fungal endocarditis is an uncommon occurrence disease carries a high risk of morbidity and mortality.In the present study we evaluate the non-invasive methods for the diagnosis of this infection in the patients with suspected infective endocarditis.

Methods: The cardiac valve, vegetation and embolic materials by surgery from the patients were examined for fungal infections by direct smear and culture.At least two blood samples were cultured by bedside inoculation into BACTEC medium. Galactomannan,mannan Ag ELISA and real-time PCR assay tests were performed on the patients' sera

Results: From 25 patients with suspected infective endocarditis, 8 cases were found to be with proven fungal endocarditis, according to the culture results. The etiologic agents were A. niger, A. flavus, C. albicansand Aspergillusfumigatus. Blood culture was positive only in one case.The sensitivity, specificity, positive and negative predictive values,positive and negative likelihood ratiofor GM test were 80%, 84%, 62%, 94%, 5, 0.2 respectively and for real-Time PCR were 87.5%, 94%, 87.5%, 94%, 14.6 and 0.1, respectively. As mannanantigen was only positive in one patient, we preferred not to calculate the sensitivity or specificity of the test.

Conclusion: According to our study, both GM antigen test and Real time PCR can serve as noninvasive and reliable tests for the diagnosis of FE, compare with culture as the gold standard test. In patients with poor condition who cannot tolerate the open heart surgery for sampling, these methodscould be useful for early detection and identification of etiologic agents.

Keywords: Galactomannan Ag test, mannan Ag test, fungal endocarditis, aspergillosis

P311: **Genetic analysis of clinical and vaccinal strains from Bordetella pertusiss by Pulsed-Field Gel Electrophoresis (PFGE)**

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Background and Aim: Whooping cough caused by Bordetella pertussis is a worldwide disease. The organism continues to circulate even in populations where high vaccine coverage of infant and children is achieved. Although, the mortality and morbidity have been substantially reduced, peak of the disease appears every 3 to5 years. Waning immunity and changing epidemiology are often referred to as explanations of this phenomenon. Pulsed-Field Gel Electrophoresis (PFGE) is used as an epidemiological tool for surveillance studies of B. pertussis strain variations over time and for identification of outbreaks isolates. PFGE provides a highly reproducible restriction profile of large bacterial DNA fragments. It is of particular interest to monitor the bacterial population and its possible influence on vaccine effectiveness after the introduction of vaccines.The aim of this study was to identify molecular and genomic fingerprinting of vaccinal Bordetella pertussis used to produce whole-cell vaccine in Razi institute and field strains by PFGE method.

Methods: DNA plugs were prepared from bacterial suspension in low melting agarose then, lysis of plugs with lysis buffer containing proteinase k and The tubes are incubated overnight in a waterbath at 55 C. plugs were washed with a suitable buffer. Finally, digested by restriction enzyme xbaI and electrophoresis performed with CEF DRII apparatus, and stained by sterile solution containing ethidium bromide. The gel was observed by gel doc apparatus(BIO-RAD model number: universal Hood II).

Results: DNA profiles obtained from genomic fingerprinting of vaccinal and circulating strains were compared. Final results will be discussed, in details later on, in the congress.

Conclusion: Due to high incidence and different circulating strains in the world, it is necessary to analyze genetic patterns of the vaccinal and circulating isolates by molecular genetics methods such as PFGE.

Keywords: Bordetella pertussis,fingerprinting ,PFGE,XbaI

P312: **Status of Cutaneous Leishmaniasis in rural communities of Varamin city, Tehran province, Iran, 2011-2013**

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Background and Aim: Leishmaniasis is one of the most important zoonotic diseases in Iran, different clinical forms, from Cutaneous to Visceral. Surveillance data indicate that the number of cases increased in recent years over the country, comprising Varamin city, Tehran province(from 18 cases in 2009 to 32 in 2011), leading the present study.

Methods: Samples were taken from skin lesions of 70 suspected Cutaneous Leishmaniasis pateints with 2 to 74 year age which reffered to the Health center of Varamin, Tehran province. Samples were collected by a sterile lancet from the swollen edge of the lesions of the cases and thin smears were prepared, fixed in methanol, stained by Geimsa and examined by microscope (100X).

Results: The leishman bodies (Amastigote) were observed in 35 out of 70 suspected patients (50%) by parasitological methods. Among them about 13 cases (37/14%) had travel to endemic area of Cutaneous Leishmaniasis like Isfahan, Kashan and so on. Most patients (91/43%) had Iranian nationality and 71/43% were male. Duration of the skin lesions was varied between 3 days to 1 years. Of all lesions, 25%, 22/5%, 17/5%, 10% were located on legs, hands, foreams and face, respectively and most of them wet sores.

Conclusion: As data show over the years there has been an increase number of Cutaneous Leishmaniasis in study area. This situation makes highly necessity of more investigation, which is leading by authors.

Keywords: Cutaneous Leishmaniasis, Leishmania parasite, Varamin

P313: **Bacterio-opsin Play a regulatory role through the E,Fand G trans-membrane domains**

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Background and Aim: Bacterio-opsin has negative control on the Bacterioruberin production by lycopen elongase in Halobacterium salinarum. This research attempted to analyze the interactions of these proteins to find interacting domains of them that contribute in the down regulation of the lycopen elongase. The last enzyme catalyzes production of the bacterioruberin by the adding of 5C units to lycopen.

Methods: The native tree dimentional structure of the bacterioopsin downloaded from the protein data bank (PDB) and the mathematical model of the lycopene longase were modeled with swiss model server(http: //swissmodel.expasy.org/) and used as inputs for the ZDOCK software (http: //zdock.umassmed.edu/software/) for protein docking computation .ZDOCK is among the best for protein docking algorithms and performs a full rigid-body search of docking orientations between two proteins; also ZDOCK uses a fast Fourier transform based algorithm to perform a global search in the translational and rotational space without the need for assumption about binding sites.

Results: Findings revealed that bacterio-opsin could bind to the lycopen elongase through 8 amino acids located in its E,Fand G trans-membrane domains.

Conclusion: It seems that these amino acids are available in the cytoplasmic form of this apo-protein. When the Bacterio-opsin bind to retinal and positioned in across of the cellular membrane and forms purple membrane the domains containing essential amino acids embed in the bilayer membrane of the the archean cell and could not bind to the related domain(s) of Lycopen elongase. So it’s no longer play regulatory role in the bacterioruberin production pathway. These results are consistent of the M. Dummer findings. Dummer et al. found bacterio-opsin prevents adding of 5C units to the lycopen and down regulate bacterioruberin production.

Keywords: Bacterio-opsin ,lycopen elongase,domains,regulatory role

P314: **The effect of propolis feed on mycological and histopathological findings and survival in old mice with disseminated candidiasis**

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Background and Aim:: Disseminated candidiasis is an opportunistic infection in aged animals by candida albicans,. Propolis is a resin component collected by honey bees from different part of plants. The immunomodulatory and antimicrobial activities of the propolis have been approved by investigators . The purposes of this study were to determine colony forming unit (CFU) of kidney and liver cultures in infected and infected /propolis groups and effect of Iranin propolis on histopathology in different tissues.

Methods: Thirty 8-month-old Balb/C mice were divided into 3 groups. Group 1 received intravenously Candida albicans (C. albicans) (2×105 cell) and orally the ethanolic extract of propolis (100mg/kg per day) for 7days, group 2 received intravenously Candida albicans (C. albicans) (2×105 cell), group 3 received normal saline (NaCl 0.9%, 0.1 mL/day) as control . After 8days of experiments, all mice were euthanized. Then, tissue samples were collected. They were taken for histopathology and qualitative and quantitative cultures.

Results:: Based on histopathological findings, the lesions were categorized in degrees 2 and 3 for the kidneys in infected / propolis group and infected group, respectively. Significant difference was observed in colony forming unit (CFU) of kidneys ($P< 0.001$) and liver ($P< 0.05$) cultures in infected and infected /propolis groups, respectively.

Conclusion:: It is suggested that propolis could inhibit the colonization and invasion of Candida in deep organs.

Keywords:: Propolis, Disseminated candidiasis, old mice, Histopathology

P315: **The Comparison of External and Cell Bound Urease Activity of Proteus mirabilis (ATCC 7002)**

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Background and Aim: Proteus mirabilis, a common agent of nosocomially acquired and catheter-associated urinary tract infection, is the most frequent cause of infection-induced bladder and kidney stones. The Enzymes urease is thought to play a major role as a virulence factor in urinary-tract infections with Proteus although it may not be the only factor

Methods: simple and precise method of detecting of urease activity is using urea test. It involves determination of the ammonium released by urease activity when bacteri is incubated at 37°C. In this study we examine the activity of proteus mirabilis urease with urease broth and urea agar method ,from two different resource: one is the cell free supernatant of proteus mirabilis culture and the other was proteus mirabilis culture as cell bound urease.

Results: Results showed that in conditions of cell bound urease, the test with urea broth and urea agar showed positive result after 30 and 60 min, and in conditions of cell free supernatant the test showed positive results after 60 and 120 min respectively.

Conclusion: It can be conclude that the most majority of urease on Proteus mirabilis is intracellular or bounded to the cell wall. According to the result the urea broth is more faster test for detecting of urease for all kind of urease resources.

Keywords: Proteus mirabilis urease, urease test, cell free and cell bound urease

P316: **Study of effect instruction Higher Education Institute of Rabe – Rashidi biologycal sciences students' attitudes towards Bioterrorism in year 2013**

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Background and Aim: Appearance of the modern biotechnology and the application of microbe microorganisms at the beginning of the new millennium led to the emergence of terrorist threats, including bioterrorism. Therefore, given the importance of combating the threats of bioterrorism and biological warfare, in the present study the biology students understanding of this phenomenon has been studied.

Methods: This descriptive study was conducted on 120 students of various fields of biology of higher education institute of Rabe – Rashidi, Tabriz, in 1392(2013). Correlation method was used in this study and measurement instruments were Likert type questionnaire of investigator.

Results: Our results showed that according to the respondent's viewpoints there is a correlation there is a significant relationship between awareness of bioterrorism and students tendency to participating in related workshops and trainings.

Conclusion: Given that students' awareness of bioterrorism and biological warfare is the main way to combat of this phenomenon, participating in workshops and training courses in trustee organizations and agencies could be a strategy to fight this ominous phenomenon.

Keywords: Bioterrorism, Biologic war, Bacteriology, Microorganism.

P317: **Correlation between IL-27 cytokine and Th-17 cells with progression of visceral leishmaniasis**

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Background and Aim: Diseases caused by Leishmania parasites are responsible for increasing health problems in large parts of the world. A major role of immune responses in the development of leishmania diseases is well recognized. IL-27 cytokine produced by MQs and DCs, plays important role in regulation of Th-17 cells. Th17 cells represent a newly described T-cell subset, characterized by production of IL-17. Th17 cells play a critical role in autoimmunity and chronic inflammatory diseases and participate in defense mechanisms against certain pathogens including L. donovani. L. donovani is the causative agent of visceral leishmaniasis(VL).

Methods: Pubmed and ISI databases were searched using leishmania donovani, kala-azar, Th17, IL-27 cytokine and RoRγT/IL-17 as keywords.

Results: Several studies have shown that elevated levels of IL-27 are associated with low RoR?T/IL-17 in VL patients before treatment. Th17 cells are linked to protection against human kala-azar caused by L. donovani. It was shown that IL-27 cytokine might suppress the function of Th-17 cells.

Conclusion: Decreasing the levels of IL-27 cytokine in human VL may serve as an important tool for protection against subsequent tissue damage by L. donovani.

Keywords: Leishmania donovani, RoRγT, IL-17, IL-27 cytokine, kala-azar

P318: **Molecular detection of AdeABC efflux pump genes in clinical isolates of Acinetobacter baumannii and their contribution in imipenem resistance**

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Background and Aim:: Multi-drug resistance due to Acinetobacter baumannii strains has become a significant challenge. Efflux pump play a vital role in the development of resistance in this bacteria. The aim of this study was to evaluate the frequency of the AdeABC efflux pump genes and its role in resistance to imipenem in clinical isolates of A.baumannii

Methods: The 56 isolates of A.baumannii were collected from different clinical specimens of Valiasr hospital in the Arak –Iran and all isolates were identified by standard biochemical tests. The Antimicrobial susceptibility patterns were determined by disk diffusion method and minimum inhibitory concentrations of imipenemin via E-test strips with and without CCCP efflux pump inhibitor were determined according to CLSI guidelines. The PCR test was used to detect the AdeABC efflux pump genes in isolates.

Results: All A.bumannii isolates were resistant to cefotaxim, ceftazidim, cefepim, cefoxitin, azteronam,piperacillin-tazobactam and ciprofloxacin, as well as all isolates were resistant to imipenem according to the results of the E-test method . Imipenem MIC with efflux pump inhibitor not reduced in all isolates and showed no differences in imipenem activity. The adeA , adeB and adeC genes were found in 100%, 100% and 96.5% of isolates, respectively.

Conclusion: AdeABC efflux system contributes to resistance to other antibiotics and resistance to imipenem has not been involved with this efflux system in A.baumanni isolates in current study and other mechanism such as carbapenemase enzymes play vital role to imipenem resistance in A.baumanni isolates.

Keywords: Acinetobacter baumannii, AdeABC efflux pump, resistance to imipenem

P319: **In vitro effectiveness of the ethanolic extract of Mentha pulegium on Trichomonasvaginalis**

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Background and Aim: Trichomonasvaginalis is a parasitic protozoan with a predilection for human urogenital tract and causative agent for vaginitis, cervicitis and urethritis in females.T.vaginalis is known asa cofactor in transmission of human immunodeficiency virus and may lead to adverse outcomes in pregnant women.The drug of choice is metronidazole however the main drawback to metronidazole is its wide adverse effect. This study was aimed to determine the effect of Menthapulegiumon Trichomonasvaginalisisolated from a female patient with urogenital complication.

Methods: Trichomonasvaginalis were cultured in the TYM medium for 24-48 h. An initial inoculum of 1x 106trophozoites/ml wasachieved. The ethanolic extract of Menthapulegium were obtained and different concentrationsof Menthapulegiumwere performed (100 µg/ml, 150 µg /ml, 200 µg /ml, 250 µg /ml, 300 µg /ml). T. vaginaliswere incubated with the mentioned concentrationsof extracts for 24-48 h along with positive and negative control. Positive and negative control was metronidazole (100 µg/ml)and culture medium, respectively. Experiments were performed in triplicate.

Results: The extract of Menthapulegium showed a remarkable trichomonocidal effect with minimal inhibitory concentration (MIC) at 300 µg/ml in 48 hour incubation periods. In the 300 µg/ml there were no viable trophozopies under the light microscopy. However, it should be mention that the concentrations 100-250 µg/ml failed to completely destroy the parasites.

Conclusion: Due to adverse effect of chemical drugs such as metronidazole use of plant based compounds could be an alternative approach for treatment of Trichomonasiasis. The present Results revealed that Menthapulegiumcould be considered as an appropriate drug for treatment of T. vaginalis. In vivo study regarding Menthapulegium is recommended in future.

Keywords: Trichomonasvaginalis, Menthapulegium,Invitro assay

P320: **Antagonistic effect of Nocardia brasiliensis PTCC 1422 against isolated bacteria from urinary tract infections**

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Background and Aim: The Nocardiae are Gram-positive, bacillary, branching bacteria which its hyphae often fragment to coccobacillary forms. They are an important part of the normal soil microflora in the world. N. brasiliensis has reported as antibiotic producer. Urinary Tract Infections(UTIS) remained as common clinical problem in both the community and healthcare-associated settings. The present study was designed to isolate bacteria from urinary tract infections and evaluate Nocardia brasiliensis PTCC 1422 antimicrobial activity against pathogenic bacteria.

Methods: The methods of this study was on the basis of determining the most common bacteria that causes UTIs, evaluation of antibiotic resistance profiles of clinical isolates and antibacterial activity of N. brasiliensis PTCC 1422 against isolated bacteria from patients with UTIs.

Results: Isolated microorganisms were included Acinetobater spp., Escherichia coli, Klebsiella pneumoniae , Serratia marcescens , Pseudomonas aeruginosa and Proteus mirabilis. Antibiotic susceptibility testing of isolates was performed using the Kirby- Bauer method with according to Clinical and Laboratory Standards Institute(CLSI) guidelines. The highest resistance rate was against Cefalexin, Cephalotin, Penicillin and the lowest rate was against Ceftriaxone and Ciprofloxacin. N. brasiliensis PTCC 1422 showed antimicrobial activity against tested microorganisms including E. coli(17 mm), K. pneumonia (15 mm), P. mirabilis (10 mm) and S. marcescens (15 mm) in Well diffusion method. The supernatant of N. brasiliensis PTCC 1422 by submerge culture was analysed with Gas chromatography-mass spectrometry(GC MS) method and phthalic acid was most fraction.

Conclusion: It has been demonstrated from this study that N. brasiliensis PTCC 1422 has a high potential for the treatment of UTIs.It may be valuable in therapeutic choice of patients such as UTI.

Keywords: N. brasiliensis -Urinary Tract Infections - Antagonistic effect

P321: **An optimized affordable DNA-extraction method from Salmonella enterica Enteritidis for PCR experiments**

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Background and Aim: In diagnostic and research bacteriology settings with budget and staff restrictions, fast and cost-effective genome extraction methods are desirable. Over the last two decades a number of different strategies have been developed to provide genomic material suitable for PCR-based and non-PCR genomic analyses. A typical property of many of gram-negative bacteria of Enterobacteriaceae is carrying heat-stable deoxyribonucleases. If not inactivated properly, cellular and/or environmental DNA nucleases will degrade genomic material during the extraction stage, and might give rise to incorrect results in PCR experiments. In the work presented here, a cost-efficient genomic DNA extraction protocol is proposed which provides genomic DNA preparation free of PCR-inhibitors, toxic chemical and suitable for PCR-experiments from S. Enteritidis.

Methods: Salmonella enterica serovar ATCC 13076 and an Iranian chicken isolate of S. Enteritidis obtained from Razi Type Culture Collection were cultured and four different DNA extraction methods were used as follows: plain boiling, Modified boiling (DNA boilates, prepared according to the Plain Boiling, then treated with proteinase K), Phenol-chloroform-isoamylalcohol and commercial DNA extraction method. crude cell extracts, proteinase K–treated templates and purified DNAs prepared by phenol-chloroform-isoamylalcohol method as well as a commercial extraction kit were subjected to the Salmonella enterica Enteritidis specific STM2 PCR.

Results: As results showed, treatment of crude cell extracts from S. Enteritidis with 0.02 µg/ml proteinase K guaranties stability of PCR products and offers an inexpensive, fast and effective DNA extraction method suitable for high-throughput laboratories. Being a further observation of the present work, treatment of DNA templates from S. Enteritidis even with low concentrations of proteinase K as little as 0.02 µg/ml seemed enough to extend stability of PCR products stored at 4ºC to at least 28 days

Conclusion: Considering the findings of this work, modified boiling would be our method of choice to prepare genomic material from S. Enteritidis as it is a very simple, inexpensive and fast technique for extraction that provides suitable material for PCR experiments. No specific laboratory expertise or equipment is required and the whole procedure can be accomplished within 30 min. This method is perfectly serviceable in laboratories with high throughput of samples.

Keywords: DNA-extraction, Salmonella enterica Enteritidis, STM2-PCR, DNase, proteinase k

P322: **3´untranslated region molecular analysis of coronaviruses from human and animals**

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Background and Aim: Coronaviruses are enveloped viruses with a large, non-segmented capped and polyadenylated single-stranded RNA genome, located in family Coronaviridae. Because of previous reports on potential of coronaviruses to infect other species such as human, understanding the genetic characteristics would be very helpful not only in differentiating strains but also in distinguishing origin of each isolate and designing an effective vaccines as needed.

Methods: In this study, 3´untranslated region(UTR) nucleotide sequence of 23 coronavirus reference strains related to different species including SARS Coronavirus obtained from NCBI(gene bank), analyzed and compared with each other using MEGA4 and Bioedit.

Results: The lowest and highest nucleotide similarity of SARS and other coronaviruses were related to bovine, murine, HCov and IBV. Based on phylogenetic tree analysis, bovine strains classified with human strains and murine strains together. SARS strains grouped together with avian coronaviruses.

Conclusion: Identifying coronavirus circulating strains and their genetic relationship is necessary as preventive strategy against coronaviruses such as causative agent of SARS. Based on our results, molecular analysis of 3 UTR nucleotides could be helpful in understanding epidemiologic characteristics of coronaviruses specially human envolved strains.

Keywords: Coronavirus, 3 untanslated region, Molecular analysis, Phylogeny, Similarity

P323: **Isolation of potentially pathogenic free-living amoebae from mucosal tissue of immunosuppressed patients in Tehran, Iran**

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Background and Aim: Ubiquitous nature of free-living amoebae (FLA) can lead to the amoebae colonization in mucosal tissue such as nasal and mouth cavity of people. Colonization of potentially pathogenic free-living amoebae in high risk people including immunosuppressed patients could be a threat for developing fatal Acanthamoeba granulomatose encephalitis (AGE). The main aim of the present research was to determine the presence of potentially pathogenic free-living amoebae in mucosal tissue of immunosuppressed patients using morphological criteria in Tehran, Iran.

Methods: Overall, 133 samples were collected from immunosuppressed patient. Oral cavity was the site of sampling from each patient. Each sample was cultured on the non-nutrient agar (NNA) with a layer of heat killed Escherishia coli. Positive plates were submitted to cloning for elimination of bacteria and fungi contamination. Purified plates were then examined for the presence of free living amoebae using page key.

Results: Of the 133 samples, 47 were showed a positive growth for free living amoebae. All of 36 samples were cloned successfully. Interestingly, 35 plates contained Acanthamoeba spp. with flat shape trophozoites and double walled cyst with star shape endocysts. Five plates contained round small cysts with wormy shape trophozoites which attributed to Hartmannella and 6 plates contained giant amoeba called Thecamoebae. It should be mention that there were several palte with mixed amoebae.

Conclusion: The presence of potentially pathogenic free living amoebae in mucosal tissue of immunosuppressed patients including Acanthamoeba and Hartmannella could be a high risk for people with impaired immunity. Developing of Amoebae-related infections in such patient is probable and monitoring of these patients is crucial for preventing amoebae related infections.

Keywords: Pathogenic free living amoebae, Morphology, immunosuppressed patient

P324: **Controlled Synthesizing Of Thin Films Single_Layer And Multi_Layers ZnO And NiO Nanocomposites Through Sol_Gel Method And Studying Their Antibacterial Performance**

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Background and Aim: Background Aim: Deactivating many micro-organisms through TiO2 optic catalyst particles with platinium was first reported by Matsunga et al. in 1985 [1].ZnO plays an important role in removing micro- organisms in self – cleaners and self sterilized materials.this can be considerably helpful in disinfecting medical devices,food prepration industry,textile industry or air conditioning filters.NiO as an absorbant with high chemical stability as well as chemical,electrolyte and optical characteristics is widely used in electrochromic devices,intelligent windows,gas sensors and it has also attracted much attention as a catalyst in electrochemical capacities of cellfuel.the present study tries to synthesize a composite system in the form of a thin film containing NiO/ZnO nanoparticles(through sol_gel method)and study its antibacterial characteristics.

Methods: Methods: The two sols were prepared separately using Zn(CH3COO)2•2H2Oand Ni(NO3)2.6H2O as precursors.Then,The glass substrateswere dipped alternatively in each sol, six coating cycles were repeated for each layer and finally thermal annealingwere performed at 500 oC.XRD pattern correspond to ZnO hexagonal wurtzite structure forall samples, Fig. 1.The photobiocideactivities of samples against a Gram-negative bacterium Escherichia coli and a Gram-positive bacterium Staphylococcus aureuswere investigated applyingthe so-called "antibacterial drop test"under UV illumination and in the dark condition.

Results: Results: Antibacterial activity of multilayeredZnO/NiO/ZnOfilm was higher compared with single film, Fig. 2. In addition, Escherichia coli inactivated more efficiently compared to Staphylococcus aureus.The compatibilities of multi-layer oxide films and the choice of substrate material for latticematching and preferential growth along certain crystallographicdirectionare the important factors for improved ZnO/NiO/ZnO film.

Conclusion: Fig. 1. XRD patterns of samples 5min 15min 30min 40min Blank Fig. 2. Images of the petriplates showing growth of bacterial colonies of E. coli using ZnO/NiO/ZnO as catalyst after 40,30,15 and 5 min UV illumination References [1] Maneerat C.,Hayata Y.,"Antifungal activity of TiO2 photocatalysis against penicillium expansum in vitro and in fruit tests",Int.J.Food Microbiol;2006,107: 99-103.

Keywords: self sterilized ,composite,thin film ,nanoparticles,sol_gel ,antibacterial

P325: **First report of clonal evolution multidrug-resistant Acinetobacter baumannii Isolates in west of Iran by Pulsed-Field Gel Electrophoresis (PFGE)**

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Background and Aim: A. baumannii are usually multidrug resistant (MDR), including third generation cephalosporins, amino glycosides and fluoroquinolone. Pulsed-Field Gel Electrophoresis (PFGE) was then used to investigate the genetic relationships among the MDR isolates. The aim of this study was to determine MDR isolates, the existence of OXAs genes and finger printing by PFGE among MDR isolates of A. baumannii collected from Kermanshah hospitals.

Methods: Eighty-four A. baumannii were collected from patient at Kermanshah hospitals and Forty-two isolates identified MDR phenotype. The isolates were identified by biochemical tests and API 20NE kit. PCR was performed for detection of blaOXA-23-like, blaOXA-24-like, blaOXA-51-like and blaOXA-58-like betalactamase genes in MDR isolates and clonal relatedness was done by PFGE (with the restriction enzyme ApaI) and patterns analyzed by Bionumeric 7.00.

Results: This study showed high resistant to ciprofloxacin, piperacillin, ceftazidime and also resistant to other antimicrobial agent and more spread blaOXA-23-like gene (93%) in MDR isolate. The PFGE method obtained 6 clones: A (10), B (9), C (5), D (4), E (11) and F (3) that clone E was outbreak and dominant in different wards of Hospitals studied. Most of isolates in clone A, B and E antibiotic resistance were higher than other clones. An isolate from the emergency ward of these hospitals had an indistinguishable isolates PFGE profile and similar resistance profile to isolates from ICU, suggesting likely transmission from ICU to emergency via patient or hospital staff contact. Only tigecycline and colistin remains effective for the treatment of infections caused by MDR A. baumannii.

Conclusion: A. baumannii isolates from the ICU and emergency at Kermanshah from March 2010 to December 2011 had high resistance rates to the majority of antimicrobials commonly used in hospitals.

Keywords: Acinetobacter baumannii, multidrug resistant, OXA-type; PFGE

P326: **In vitro antibacterial activity of defensin protein against Gram-negative and -positive bacteria.**

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Background and Aim: Defensins are a major group of antimicrobial peptides and are found widely in vertebrates, invertebrates and plants and naturally are an essential component of the innate immune system of multicellular organisms. These small peptides have different types that are active against a broad spectrum of microbial agents including: Fungi, bacteria, viruses, yeast and etc. in this study we investigated in vitro antibacterial activity of recombinant defensin protein against Gram-negative and -positive bacteria.

Methods: Recombinant defensing protein is produced by gene sd2mod cloned with marker gene (LicBM2) in pBISN1-IN plasmid by transient expression using agroinfiltration technique. The Active protein level of extracted proteins, was measured by glucose decreased test (based on activation of β1,3-1,4gluconase enzyme, produced by LicBM2 gene).To determine antimicrobial properties of this protein, we investigated the growth of E. coli (a gram-negative bacterium) and S. aureuse (a gram-positive bacterium) in the presence or absence of defensing protein. We used the disk diffusion method and the orythromycin antibiotic as positive control and control leaf protein extraction and trisHCl as negative controls.

Results: The results show that recombinant defensin protein has inhibitory effects on E. coli and S. aureuse. The growth and division of bacteria around the disks containing protein, is very slowly. This inhibitory is more against gram-positive bacteria S. aureuse.

Conclusion: Genetic engineering of plants expressing this gene has been developed as a means of creating disease resistant plants with an ability to kill a broad spectrum of microbes. Furthermore, it is believed that this protein are less vulnerable to the development of microbial resistance as they do not interfere with microbial biochemical pathways like conventional antibiotics, but instead physically disrupt microbial membranes.

Keywords: Defensin, Gram-negative and -positive bacteria, transient expression, agroinfiltration

P327: **Effect of Zataria multiflora Boiss. Essential oil on the growth of Lactococcus garvieae and Streptococcus iniae in fillets of Oncorhynchus mykiss**

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Background and Aim: Plant essential oils and their components are perceived to exhibit antimicrobial activities. In this study, the antibacterial effects of essential oil extracted from Zataria multiflora Boiss, were evaluated against Lactococcus garvieae (Ir-170A(856bp)) and Streptococcus iniae (GQ850377) in fillets of Oncorhynchus mykiss.

Methods: Minimum inhibitory concentration (MIC) and Minimum bacterial concentration (MBC) with essential oils were measured by the broth micro dilution method. MIC and MBC of essential oil for L.garvieae 7.8 and 15.6 µg/ml, but for S. iniae 62.4 and 250µg/ml were determined.

Results: The results showed, the essential oil antibacterial effects against two bacterial. The study has also demonstrated that all concentrations of Z.multiflora Bioss were effective against L.garvieae and S.iniae. However, with the value of t at 0.405%, the effectiveness was 71.91%. This value had the biggest effect on L.garvieae at 4 0C, but for S.iniae, the higher concentrations of essential oil did not showed a statistical significant different (P>0.05) in bacterial control

Conclusion: Thus, essential of Z.multiflora with high antibacterial activity selected in this study could be a potential source for inhibitory substances against some food-borne and zoonotic pathogens and they may be candidates for using in foods or food-processing systems.

Keywords: Zataria multiflora Boiss, Lactococcus garvieae, Streptococcus iniae, Oncorhynchus mykiss

P328: **Effect of ethanol extract Tanacetum parthenium on Trichomonas vaginalis in vitro**

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Background and Aim: Trichomonas vaginalisis a flagellate parasite causing vaginosis which is a common sexual transmitted disease. Metronidazole is the drug of choice for this disease but due to its side effects it is necessary to search for an alternative drug. So in this study the effect of Tanacetum Partheniumon Trichomonas vaginalis has been investigated.

Methods: Hydro-alcoholic extracts of the two herbs were prepared. The extracts were dried using vacuum rotary evaporator and then they were used for in-vitro experiments.

Results: in concentrations of 4, 5, 8 and 10mg/ml of Tanacetum Partheniumthe parasite didn’t grow, and the effect of the extracts on Trichomonas vaginalis was similar to the effect of metronidazole on the parasite.

Conclusion: Tanacetum Parthenium has efficient effect against Trichomonas vaginalis growth in culture medium and so these two herbs can be considered as alternatives for methronidazole.

Keywords: Tanacetum Parthenium, Trichomonas vaginalis, hydro-alcoholic extracts

P329: **Serologic molecular parasitologic evaluation of ocular toxoplasmosis in patients referring to ophthalmology centers of Farabi, Labbafinejad and Imam Hossein hospitals**

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Background and Aim: Toxoplasmosis is one of the most prevalent parasitic infections zeotoze or common between human and different animals. Its worldwide expansion makes this parasite so important. People suffer from the disease either acquired or congenital. Toxoplasma gondii is considered as an opportunistic and the most dangerous infection in immunusuppressed individuals or pregnant women. The parasite reaches to the eye and its retina through circulation. After that, the parasite goes to the choroid and affects its neuronal layers. Toxoplasmosis is known as the most common causes of chorioretinitis worldwide. Attacks to the eye could be chronic and sometimes infection relapses. In this case, the damaged tissue which is associated with the eye neural layers such as retina and choroid would not repair. Emergence of clinical symptoms in patients is related to the age, the injured site and cyst size. An injury can remain active about four months and often the site of injury is in the posterior pole or proximal to it. The posterior pole scares can persist a serious threat for the vision throughout one's life. Serologic and molecular tests are carrying out complementary for recognition of acquired infection or infection relapse. The aim of the current study is to represent efficient and effective laboratory methods for precise and quick diagnosis of ocular toxoplasmosis.

Methods: In this study, sampling had done from 30 major ophthalmology centers in Tehran, Farabi, Labbafinejad and Imam Hossein hospitals. The referring patients to these centers who were suspicious to ocular toxoplasmosis examined and their clinical symptoms recognized by the specialists. They also filled out a cooperation consent form and blood sampling taken for our toxoplasmosis thesis. The samples then transferred to the parasitology laboratory of Shahid Beheshti University of Medical Sciences. Thereafter, the serum was assessed for antibody titers of IgM and IgG by ELISA kit. Buffy coat of the samples was employed for DNA extraction and molecular PCR test. For performing the PCR primer of the gene was checked. Tissue, BAG1, the needed material and Amplicon Master Mix were used.

Results: From the 52 blood samples, 20 patients (38%) were positive for ocular toxoplasmosis that from them 8 patients (40%) had positive IgG+ and IgM+and 12 patients had positive IgG+ and IgM- titers. From the 52 patients suspicious to ocular toxoplasmosis 20 patients (38%) had positive form of the disease in serologic test (ELISA) as follows: 8 patients (15%) showed positive titer IgG+ and IgM+ for the disease and 12 patients (23%) demonstrated IgG+ and IgM-. Using the molecular method (PCR), 18 patients (34%) were determined to have the positive form of the disease.

Conclusion: Serologic and molecular tests are carrying out complementary for recognition of acquired infection or infection relapse. The aim of the current study is to represent efficient and effective laboratory methods for precise and quick diagnosis of ocular toxoplasmosis.

Keywords: Toxoplasmosis-immunusuppressed-ocular

P330: **Preparation and evaluation of monoclonal isolates from Leishmania major inducing delayed-type hypersensitivity reactions comparable with leishmanin**

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Background and Aim: Leishmania major is a pathogenic intracellular protozoan parasite that cause a wide spectrum of cutaneous infections with clinical outcomes ranging from self-healing cutaneous lesions to disseminated and non-healed ulcerations. Cell-mediated immunity (CMI) plays an important role in resistance against L. major infection. Delayed-type hypersensitivity (DTH) reaction detected by leishmanin skin test is a practical method for evaluation of CMI and is widely used for epidemiological assessment of exposure to leishmanial antigens, evaluation of vaccine candidates and as adid for diagnosis. Skin testing in leishmaniasis, generally known as Montenegro or Leishmanin test, requires a standard, pure and homogenous antigen. Preparation and evaluation of mono clone isolate from L. major for skin testing.

Methods: Efforts were carried out to isolate monoclones /ER) by limiting dilution57from L. major reference strain (MRHO/IR/ assays. The isolated clones were evaluated in vitro using SDS-PAGE and lymphocyte transformation tests (L.T.T), using peripheral blood mononuclear cells of healed individuals from L. major infection. The potency of the clones were evaluated by skin testing in four immunized guinea pigs against L. major. The isolated monoclones along with leishmanin were injected intradermally in shaved abdominal area of animals.

Results: The results indicated that three out of seven clones, (ie. clone B, C and D) could exibit a single band in SDS-PAGE analyses. These monoclones were used for evaluation by LTT which all exhibited comparable stimulation indices respectively) with ,31.2 ± 34.8 and 56.2 ± 17.8 ,30.2 ± 17.8( ). In vivo evaluation of monoclonal isolates in 215.3 ± 01 (leishmanin guinea pigs revealed that all three monoclones showed similar indurations in comparison to leishmanin. In addition, clone C showed a mm) than leishmanin905.0 ± 333.8significantly higher indurations ((4.44 ± 1.57mm) in guinea pigs tested with undiluted antigen C (p=<0.05).

Conclusion: The data indicated that the isolated mono clones have the ability to induce comparable reactions in vitro and in vivo with standard leishmanin and might be used as suitable purified and homogenous antigens for skin testing in leishmaniasis studies.

Keywords: monoclonal isolates, Leishmania, delayed-type hypersensitivity reactions, leishmanin

P331: **Antifungal producing organisms from soil community: versatile weapons for control of pathogenic fungi**

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Background and Aim: Invasion of humans and animals with pathogenic fungi from the different genera is an important problem that is not yet under adequate control despite modern antifungal compounds production technologies. Among the existing biodiversity, bacteria have received major consideration not only for their extremely distribution and population diversity, but also for their capability to produce a wide array of bioactive metabolites with antimicrobial properties. Nowadays, hundreds of chemically diverse antifungal compounds have been isolated from a vast array of bacteria and there are more compounds waiting to be discovered by researchers yet.

Methods: Rapid screening of a large number of bacteria from soil community against some pathogenic fungi was achieved by visual agar plate assay. Aside from morphology, PCR-based molecular techniques was very suitable for confirmation of identity of isolated bacteria at the genus and species level. Antifungal compound of the bacterium was partially purified from culture filtrate through a purification scheme of methanol extraction, Diaion HP20 ion-exchange chromatography, thin layer chromatography (TLC) and HPLC techniques.

Results: Molecular identification of the bacterial strains based on 16S rDNA sequence analysis indicated that the bacterial isolates were mainly belong to the genera of Bacillus (79.5%), including B. subtilis , B. amyloliquefaciens , Pseudomonas (0.8%), Acinetobacter baumannii , and Actinomycete spp . Based on the visual plate assay results, B. subtilis isolate (BS-45 strain) showed the most remarkable growth inhibition and strong antifungal activity against tested fungi.

Conclusion: Antifungal bacteria to be considered not only as potential candidates for biological control programs, but also for finding rich sources of useful metabolites with potential application in antifungal drug discovery. Here we describe a practical approach for isolation and identification of antifungal bacteria from soil and how we can characterize their bioactive metabolites by novel well-developed techniques.

Keywords: Antifungal activity, Soil organisms, Pathogenic fungi, Purification of antifungal compounds

P332: **Design of a Lambda Bacteriophage as a Herceptin delivery vehicle to Her2 antigen expressing carcinoma cells**

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Background and Aim: Progress in the field of antibody engineering has led to the production of improved humanized antibodies. The maintenance of antibodies activity and inhibition from their degradation is an important method to improve the antibodies efficiency. The aim of this study was to produce Lambda-bacteriophage that conjugated by Herceptin monoclonal high affinity antibody as a vehicle towards HER-2 expressing breast carcinoma cells. Bacteriophage λ is a temperate phage with inherent biological safety in mammalian cells.

Methods: We designed λ-phage particles containing a surface Herceptin antibody. Herceptin was purchased and conjugated on the lambda protein surface by chemical method. The conjugated phages were selected by biopanning and confirmed with competitive ELISA assay. The SKBR-3 and human bone marrow stem cells were treated by the carrier λ phages. The cell growth inhibition assay was performed by MTT Cell Viability Assay Kit.

Results: After the 4th round of biopanning there was a significant enrichment in the phages specifically binding to the antigen. The ratio of targeted phages increased in the fifth round. The ? bioparticles significantly inhibited the proliferation of HER-2 positive SKBR-3 cells.

Conclusion: Bacteriophage λ could be used efficiently as a Nanobioparticle for delivery of multivalent antibodies to mortify HER-2 positive breast carcinoma cells as a nanomedical therapeutic.

Keywords: Bacteriophage, Cancer, Delivery, Lambda particle

P333: **Immunological Evaluation of S.areus Cp8 Conjugate to Tetanus toxoid in mice as a Vaccine Candidate**

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Background and Aim: Conjugation S.aureus Capsular type 8 with tetanus toxoid (TT) as a protein unit can causes a high long and stable protective vaccine in adults and infants . also this vacci can actives T – helper lymphocyte and it makes memoral cells.

Methods: Cp8 extracted by enzyme digestion, then dialysis .To improve immunogenicity, the purified antigen was conjugated to TT with ADH as a spacer and EDAC as a linker. The reaction mixture was passed through a sepharose CL-2B column. Sterility and toxicity tests were shown that prepared conjugate was non-toxic and non-pyrogenic. Then four groups of female BALB/c mice (each group was included 15 mice) was selected. Vaccination was performed by three doses with two week interval. Then serum samples was collected and antibody responses against Cp8 was measured by ELISA method for total IgG, IgG1, IgG2a, IgG2b, IgG3, IgM and IgA. Two week after first dose of vaccination there was no significance different antibody titers between groups that were immunizided by Cp8 and Cp8-TT. But after second and third doses, Cp8-TT showed significance increasing in all types of antibodies titers in versus Cp8.

Results: Overall results of anti Cp8 inductions for total IgG, IgM, IgA, IgG1, IgG2a, IgG2b, and IgG3 were shown: Cp8-TT> Cp8 >TT. The anti Cp8 IgG antibody was predominantly of IgG1 subtype and then IgG3, IgG2a, IgG2b, respectively.

Conclusion: Cp8 from S.aureus increase anti Cp8 antibodies in conjugate form with tetanus toxoid and can be an appropriate effective candidate vaccine for this bacteria.

Keywords: S.aureus , Capsular type 8 , Tetanus toxoid , conjugate, ELISA

P334: **Prevalence of Iintestinal Parasites in Ilam City, 2012, Iran Survey**

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Background and Aim: . Prevalence of parasite a function of health conditions the incidence of infection is most in tropical and subtropical areas. But the distribution returns to economic –social condition. Aim: The purpose of this study was review prevalence of intestinal parasite in the city of Ilam.

Methods: this cross-sectional study was conducted in Ilam city, 300stool samples (150mals, 150females). Were collected and survey to direct, expansion and formalin-ether assay were analyzed.

Results: Finding of this study showed, that of the 300stool samples, 180samples were infected were diagnosed. Than 180 samples, 93samples infected with the parasite giardia(35.5),55samples infected with Entomoeba Histolytica (21.8)and 32 cases infected with Ascaris (12.7),respectively. In this study, were more pollution men to women (p<0/1)

Conclusion: The results of this study showed the prevalence Giardia lamblia is considered one of health problem in Ilam. There fore is recommended for future occurs more studies in filed.

Keywords: prevalence, Intestinal parasite, Ilam

P335: **Evidence for the presence of Hyaluronat lyase production in Pseudomonas sp. LA_03 isolated from pathological specimen**

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Background and Aim: Hyaluronic acid degradating enzymes(HAases) are present in mammals, insects, parasites and bacteria. HAases especially Bovine testicular hyaluronat lyases(BTH) preparations are needed in many fields such as orthopaedia, oncology, ophthalmology, dermatology, surgery, internal medicine, gynaecology etc. due to depleted supplies of pharmaceutical BTH preparations, a number of cases of iatrogenic strabismus have been observed after cataract surgeries. With respect to this shortage, we aimed finding a bacterium with high capacity of HAase production and will suggest pharmaceutical preparations with this bacterium as replacement of BTH.

Methods: The pathological samples were collected and transferred into blood agar medium. HAase producing capabilities by measuring reduction in turbidity and hydrolyzed zone of substrate hyaluronic acid were performed and then partial 16S rDNA gene sequencing was applied to identification bacterial isolates with high level HAase production

Results: A BLAST search of the 16S rRNA sequence against NCBI, was showed that a promising isolate was from genus of Pseudomonas. Pseudomonas sp. LA_03 with the high HAase production from abscess will select for further investigation.

Conclusion: High production of HAase by this promising isolate, made Pseudomonas sp. LA_03 the suitable candidate for biotechnology, commercial, pharmaceutical purposes and industrial application.

Keywords: Pathological specimens, HAase, Pseudomonas sp. LA_03

P336: **Study the visceralization pattern of Leishmania tropica in BALB/C mice**

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Background and Aim: Leishmania tropica (L. tropica) is one of the causative agents of cutaneous leishmaniasis. This parasite may involve visceral organs, such as spleen, as well as skin. Visceralization is an important mechanism in pathology of the diseases caused by Leishmania parasites. Thus study the factors responsible for visceralization of L. tropica has implications in prevention and treatment of the diseases caused by this parasite. We investigated the role of parasite infective dose on the visceralization pattern of L. tropica infection in BALB/C mice.

Methods: The parasite was cultured in NNN medium. When the parasites reached to stationary phase, two different doses (high dose: 106 parasite, 103 parasite) were prepared and injected into BALB/C. The parasite load were determined in different tissues of the mice, using limit dilution method, one week, one month and four months after infection.

Results: The results showed that at one week post-infection, parasites at high dose and low dose entered the local lymph node but parasites of none of the mentioned doses entered spleen. At one month post-infection, parasites of both high and low doses entered the local lymph nodes, while parasite at the high dose but not the low dose entered the spleen. Finally after 4 months, the results were exactly the same as those at one month post-infection.

Conclusion: The result indicated that high dose of L. tropica visceralizes in BALB/c mice.

Keywords: Leishmania tropica, Visceralization, Parasite load

P337: **Quantitative and qualitative analysis of the antilisterial activity of the cell-free culture of Enterococcus facium**

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Background and Aim: Enterocins are peptides with antimicrobial activity synthesized by Enterococcus species, such as E.faecalis, E.faecium, E.durans and E.munditi. E.faecium can produce enterocins, which are various, having great diversity in their structure, and are active against numerous microorganisms, especially food borne pathogens and against microorganisms of environmental and medical interests. These studies pointed out the distribution and importance of E.faecium as sources of enterocin. Many of these enterocins show strong bacteriocide activity against microorganisms such as Listeria monocytogenes. Enterocins are small, heat stable. This study was designed to characterize the bacteriocins produced by E. facium for their antilisterial activity spectrum, evaluating their antimicrobial activity measured in Arbitrary Units (AU/mL), define optimum pH and optimum temperature for enterocin production.

Methods: To determine the optimum temperature for bacteriocin production, we used 10ml of MRS broth with different pH (4to8) was inoculated with 0.5ml of an overnight culture and incubated at 30,37 and 40 °C. Samples were collected after 24h and examined for bacteriocin production as described later. The strain of the selected E.faecium which showed the highest inhibition zone for Listeria monocytogenes during the primary screening were used for further studies. Cell Free Supernatant (CFS) from the strain was obtained and its inhibitory activity against Listeria monocytogenes was assayed by the agar well-diffusion test. CFS was obtained from MRS broth cultures after 24 hr incubation at 37ºC by centrifugation at 7500 g for 10 min at 4ºC. To ensure sterility, the supernatant was filter through 0.45-µm pore-size filters and heated for 25 min at 70 ºC. Ammonium sulfate was added to the supernatant. The protein precipitate was pelleted by centrifugation at 10,000 × g for 20 min and dissolved in 500 ml of 20 mM sodium phosphate buffer (pH 5.0). The supernatant was transferred to a clean sterile container.

Results: The optimum temperature for the production of bacteriocin obtained to be 37°C and the bacteriocin activity at this temperature was higher than that observed at 30 and 40°C. The optimum pH for the production of bacteriocin was 6.8 and the bacteriocin activity at this pH was higher than that observed at other conditions. The antibacterial activity of crude enterocin produced by E. faecium was tested on Listeria monocytogenes. The zone of antilisterial inhibitory was 19 mm and protein content of the crude enterocin of E. facium was 0.294 mg/ml and its activity was 20 AU/ml.

Conclusion: This study let us think of the fact that the enterocin produced by our E. faecium isolated from vaginal swab had antilisterial activity. This study has revealed that the enterocin produced by the E. faecium from the vaginal swab has a wide antibacterial spectrum and it is stable for 25 min at 70 ºC. It is a good candidate for medical and industrial applications. Further investigations of this claim are hereby recommended.

Keywords: entrocin, entrococci, antilisterial

P338: **Inhibitory effect of gram negative bacteria,Pseudomonase aeroginosa ptcc: 1430 as a biocontorol agent of fungi**

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Background and Aim: Biocontrol is a new technology to prevent or stop bacterial ,viral or fungal diseases.The present study was designed to investigate potential biological agent for biocontrol of saprolegniasis in persian sturgeon eggs (Acipenser persicus) by Pseudomonase aeroginosa (PTCC: 1430) in sturgeon hatcheries.

Methods: for invitro tests,prepared purified fungi from eggs.then bacteria grown in PDB media and prepared 103 to 107cfu.ml-1 concentrations.fungi challenged with bacteria in prepared concentrations in petridishes. for invivo challenge,fifty eggs were confined within Nylonic cages.Fish eggs were treated by three duration times (15, 30 and 45 min), three concentration levels of bacterial bath. (105, 106 and 107 cfu. ml-1) and two times (once at first incubation day and three times during the incubation period). Infected eggs were assessed, counted and picked up from cages daily.

Results: The result of invitro challenge revealed that the concentration of 107cfu.ml-1 inhibited the growth of saprolegnia.Increasing the growth and diameter of colony started in plates containing 106 concentrations and continued to 103 cfu.ml-1 the result of invivo challenge showed that all three concenterations can reduce the fungal infections of eggs.the best results belonged to treatments of 105 in 30and15 min. duration and one time treating.

Conclusion: The ability of bacteria to inhibit saprolegnia infection might be implicated to its ability to liquefy gelatin of such fungi (Holt et al, 1993). Another candidate for the inhibitory activity for saprolegnia is cellulose (Hussein and Hatai, 2001). Bly (1996) reported that inhibition of saprolegnia by bacteria not related to the secretory substance but rather the result of competition (Bly et al, 1996) (Bly et al, 1997). In the present study, with increasing the concentration of bacteria, the effect of bacteria to control of fungal infections was reduced in 107cfu.ml-1 that could be related to extra cellular enzymes that might damage the egg membrane.

Keywords: Pseudomonas aeroginosa,saprolegnia,biocontrol,persian sturgeon eggs

P339: **Evaluation of alpha-amylase-producing fungus Rhizopus oryzae PTCC 5263**

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Background and Aim: Rhizopus oryza produces a filamentous fungus is the class Zygomycetes archial order for the species to produce various enzymes amylolytic of which alpha-amylase enzyme is important in the economy.This enzyme is a random endo-hydrolase glycoside links in starch hydrolysis and oligosaccharides compounds with the alpha position and brings in numerous sizes.

Methods: A standard strain of the Rhizopus oryzae PTCC 5263 that the central collection of fungi and bacteria of Scientific and Industrial Research of Iran, in order to evaluate the in vitro production of the enzyme alpha-amylase was provided. Thus Purpose in vitro of the fungus in culture medium Potato Dextrose Agar The next step in designing a chemically defined medium in vitro production of alpha-amylase enzyme in these strains was investigated. To evaluate the ability of enzymatically strain PTCC 5263, starch hydrolysis test was performed. The enzyme activity by iodine method - iodine solution was measured in nm 660.

Results: Macroscopic observation of colonies initially white cotton and then wanting to gray and finally black, gray mm 8-5 to reach the height was seen. Microscopic examination of the absence of transverse walls, containing rhizoid, stolons and sporangiospores were observed under a microscope.

Conclusion: In vitro evaluation, macroscopic and microscopic observations in a semi-quantitative and quantitative strain PTCC 5263 total could Pick a chemically defined medium to produce the enzyme alpha-amylase. The enzyme production process can be commercialized.

Keywords: Rhizopus oryzae PTCC 5263, alpha-amylase, sporangiospores, Lactophenol cotton blue.

P340: **The use of indigenous and cheap carbon resources (wheat bran) in the production of Erythromycin by fermentation method.**

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Background and Aim: Biotechnology refers to production of organic products by means of their producers (microorganisms) .It means that very strategic and expensive products can be produced and synthesised by microbal population in doubling time of less than 5-6 hours

Methods: in this study the researcher worked on production of the macrolide antibiotic erythromycin by its producer strain, namely Saccharopolyspora erythreaea, in order to use indigenous and cheap carbon resources, in this study the researcher used wheat bran as a suitable carbon and energy resource in formulation of fermentation media, instead of dextrin. During the fermentation process, the influence of parametrs such as PH, microbal growth control (with no secondary pollution), and packed microbal weight (PMW) are studied and at last, amount of produced erythromycin is measured by Spectrophotometry.

Results: According to the results, the optimum concentration of wheat bran for producing this product is 33 gr/lit (replacing 50% wheat bran equivalent of 33 gr/lit, such amount, according to analysis of wheat bran and its carbohydrate contents is equal to 20 gr/lit dextrin) which is obtained on the sixth day of the fermentation process (20gr/lit dextrin + 33 gr/lit wheat bran). The comparison results for control samples (samples which contain dextrin as carbon resource and lack wheat bran) with samples that contain wheat bran, showed that, by using wheat bran, the amount of the produced erythromycin was increased and the necessary cost for its production was significantly decreased, in terms of supplying carbon and energy resource

Conclusion: using use wheat bran in the fermentation media of saccharopolyspora is considered to be a reasonable alternative compared to other carbon resources

Keywords: erythromycin, fermentation, wheat bran, Spectrophotometry

P341: **Typing of Clostridium perfringens isolated from cecum and jejunum of diseased broiler chicken by Multiplex PCR**

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Background and Aim: Clostridium bacteria are gram-positive, anaerobic, and spore forming. Clostridium perfringens are found in soil, water and digestive system of animals and human. This bacterium is classified into five different types. This bacteria produce vary toxins that can cause a variety of clinical and subclinical disease in humans and animals. There are various methods for the bacterial identification, many are labor-intensive, time-consuming, expensive and also present low sensitivity and specificity. The purpose of this study was Typing of Clostridium perfringens isolated from the different part of intestinal tract (cecum and jejunum) of broiler chicken by Multiplex PCR.

Methods: In this study, 30 feces samples from the gastrointestinal tract of diseased poultry (15 samples from cecum and 15 samples from jejunum) from different poultry house of Kerman province were randomly selected. After preparation and cultivation samples, gram staining of pure colonies was done. Bacteria were identified by biochemical tests as described in Bergey's manual. The biochemical tests including carbohydrates fermentation (sucrose, glucose, lactose and maltose), lecithin hydrolyzed, litmus milk reaction (stormy reaction), catalase test, gelatin hydrolyzed, indole produce test and motility test were carried out to identify the Clostridium perfringens. DNA extracted (by boiling in STET Buffer) from isolated bacteria for genotyping was tested by multiplex PCR with specific primers. Based on length of synthesized fragments by PCR, toxin types and bacterial strains were detected.

Results: In this study from 80% of the samples, Clostridium perfringens were isolated. All C isolated types were diagnosed as Type A. There were any differences between isolated samples of cecum and jejunum.

Conclusion: Type A is an important pathogen in broilers and is a major Food poisoning for human health. Isolation of Clostridium perfringens Type A from broiler chicken Illustrated the importance of this type of bacteria in poultry.

Keywords: clostridium, biochemical test, Multiplex PCR

P342: **Effectiveness of Thymbra Spicata Alcoholic Extract on Blood Lipid Profile Compared to That of Lovastatin in Male Rats**

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Background and Aim: BACKGROUND: Using lipid lowering agents is a usual medical procedure for prevention of cardiovascular diseases.Despite the side effects of such drugs, finding the plants with the same effectiveness will be useful. Aim: The purpose of this study was to compare the effect of Tthymbra Spicata Alcoholic Extract withthat of lavostatin on blood lipid profile.

Methods: In this experimental study, 50 adult male Wistar rats (330±20 gr) were used. The rats were randomly parted into 5 groups of 10. Groups 1, 2, 3, 4, and 5, which were, respectively, fed with normal diet, high cholesterol diet (%1 cholesterol), high-cholesterol diet with lovastatin (10 mg/kg), normal diet with alcoholic extract of thymbra spicata (100mg/kg) and high-cholesterol diet plus alcoholic extract of thymbra spicata for one month by Gavage method. At the end of one month, the animals were unconscious and the blood samples were directly taken from their hearts. Cholesterol serum concentration, LDL, VLDL, TG, HDL and CHO/HDL, TG/HDL, LDL/HDL were measured and compared among the five groups to determine the blood lipid profile. To analyze and assess the mean of evaluated parameters in terms of statistical framework, a whole randomized approach was applied using SAS.Ver.9 software.

Results: The results showed that using alcoholic extract of thymbra spicata alone (62.6±1.78), compared to the control group (71.93±1.64), caused a significant decrease in the serum level of cholesterol ($p<0.001$),TG (52.02±1.34 vs. 72.48±1.3) ($p<0.001$), LDL (6.79±1.58 vs. 16.66±1.73) ($p<0.001$), VLDL (10.36±1.15 vs.14.27±0.48) ($p<0.001$), TG/HDL (0.98±0.04 vs. 1.74±0.1) ($p<0.001$), CHO/HDL (1.18±0.05 vs. 1.73±0.09) ($p<0.001$) and LDL/HDL (0.13±0.03 vs. 0.4±0.06) ($p<0.001$), while there was a significant increase in HDL level (53.8±1.52 vs. 41.61±2.61) ($p<0.001$). Among the groups receiving high cholesterol diets, no significant differences were observed, despite taking in the extracts of Thymbra Spicata and Lovastatin.

Conclusion: According to the results of this study, not only did alcoholic extract of thymbra spicata improve lipid profile, but it could also, like lovastatin, lower blood lipids level.

Keywords: Thymbra spicata, Cholesterol, Lovastatin.

P343: **Evaluation of Antibactrial and Cytotoxic effects of aerial parts of Stachys pubescence from Iran**

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Background and Aim: Introduction: The genus Stachys belongs to the plant family of Lamiaceae . This family is well represented in the flora of Iran, at least with 200-300 species in the world and 34 species in Iran. There are some reports about pharmacological activities of this genus including anticancer, antibacterial and antioxidant effects. Aim: In the present study the antibacterial and cytotoxic activites of Stachys pubescence was evaluated against a range of gram positive and gram negative bacterial strains, cancer and normal cell lines, respectively

Methods: Methods: n- Hexane, dichloromethane(DCM) and methanol extracts of aerial parts of Stachys pubescence were prepared and their antibacterial activity against some gram negative bacterial strains as well as gram positive bacterial strains and cytotoxic effects on MCF7 (breast cancer) ,HT29(colon carcinoma), A549(human lung adenocarcinoma epithelial) as cancer cell lines and HUVEC as normal cell lines were investigated using paper-disk agar diffusion method and MTT assay respectively

Results: Results: methanol and dichloromethane extracts of S.pubescene had no antibacterial effect against none of the examined strains but n-hexane extract had a moderate activity on some bacteria especially within the gram positive group. Furthermore methanol extract had moderate cytotoxic effect on HT29 cell line. also it had more significant effect on MCF 7 cell lines in various concentrations.DCM extract only had significant effect on MCF cell line. None of the extracts had cytotoxic effect on HUVEC cell lines.

Conclusion: Conclusion: The results indicate that n-hexane extract of S.pubescene as a non-polar extract had significant antibacterial effect on the examined gram positive bacterial straines. furthermore methanol and DCM extracts were showed cytotoxic effects against specific cancer cells and non toxicity to normal cells.

Keywords: Stachys pubescence, Antibacterial activity, Cytotoxic activity

P344: **Evaluation of Antibactrial and cytotoxic activities of aerial parts of Artemisia scoparia and A. Spicigera, Asteraceae**

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Background and Aim: introduction: The genus Artemisia L., one of the largest and most widely distributed genera of the family Asteraceae, comprises about 200-400 species of herbs and shrubs, the most notable being A. annua for producing the antimalarial drug artemisinin .This genus, with its common Persian name "Dermane" and the common English name "Wormwood", includes approximately 34 species that are found wild all over Iran. There are some reports about pharmacological activities of this genus including antimalarial, antiviral, antitumor, antipyretic, antibacterial, anticoagulant, anti-inflammatory and antioxidant properties. Aim: In this study the extracts of two medicinal plants of this genus, Artemisia scoparia Waldst. & Kit. And A. spicigera C. Koch, were evaluated for their antibacterial and cytotoxic activities against a range of gram positive and gram negative bacterial strains, cancer and normal cell lines, respectively

Methods: Methods: n- Hexane and methanol extracts of aerial parts of Artemisia scoparia Waldst. & Kit. And A. spicigera C. Koch, were prepared by soxhlet apparatus and their antibacterial activity were evaluated against some gram negative bacterial strains as well as gram positive bacterial strains by using paper-disk agar diffusion.Furthermore cytotoxic effects on HT29(colon carcinoma), A549(human lung adenocarcinoma epithelial) as cancer cell lines and Huvec as normal cell lines were investigated by MTT assay.

Results: Results: Only n - Hexane extract of A-spicigera had no antibacterial effect against none of the examined strains but methanol extracts of both plants and n-hexane extract of A.scoparia had a moderate activity on some bacteria especially within the gram positive group. Furthermore methanol extract had moderate cytotoxic effect on HT29 and A549 cell lines in various concentrations.N-hexane extract had more significant effect on both cell lines. None of the extracts had cytotoxic effect on HUVEC cell lines.

Conclusion: Conclusion: The results indicate that methanol extracts of both plants as a polar extracts and n-hexane extract of A.scoparia had significant antibacterial effect on the examined gram positive bacterial straines. Also N-hexane and methanol extracts were showed cytotoxic effects against specific cancer cells and non toxicity to normal cells.

Keywords: Artemisia scoparia, Artemisia spicigera, Antibacterial activity, Cytotoxic activity

P345: **Isolation and charactrization of acid lactic bacteria from the rumen of Mehraban sheep**

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Background and Aim: Lactic acid bacteria (LAB) are an important member of microorganisms in the forestomach of ruminants. Consumption of readily digestible carbohydrate increases the number of LAB, and eventually lactate concentration in the rumen fluid. Lactate is consumed by another group of bacteria so-called lactate utilizers which their numbers are in balance with lactate producers. Overproduction of lactate decreases ruminal pH, and causes some metabolic disorders such as ruminal acidosis.

Methods: Rumen fluid was collected from three ruminally fistulated mature Mehraban sheep. The rumen fluid was filtered through four layer of cheese cloth and then 1 ml of strained rumen fluid, was added to 9 ml of anaerobic dilution solution (ADS) for serial dilution up to 10-10. Aliquots of obtained ADS from three latest dilutions (i.e., 10-8, 10-9 and 10-10) were inoculated into Hungate tubes containing anaerobic MRS and molten agar and incubated in 39˚C. After two days, individual and distinct colonies were picked up and transferred into tubes containing anaerobic MRS broth media. The tubes were incubated in 39˚C for two days again. Sub-culturing of bacteria repeated several time until the obtaining the pure cultures. Purity of isolates were confirmed using light microscopy. Morphological characteristic were studied with the gram staining method. For identification of isolates, DNA was extracted using boiling and cooling of isolates. The extracted DNA were amplified using PCR technique. RFLP technique was used for testing the genetic homogeneity of isolates at which the extracted DNA was digested using Dde1 and Alu1 restriction enzymes. PCR products were the representative of 16s rRNA gene, and sequenced via Bioneer company (Seoul, South Korea). The sequences of nucleotides were submitted to Genebank (BLAST) to identify the closest relative of isolates.

Results: Most of the isolates were rod shape and some of them were coccus, gram positive bacteria with ability of fermenting carbohydrates such as glucose, sucrose, maltose and lactose. They had no catalase activity, but able to produce indole. None of them fermented protein. The closest relative of isolates were Lactobacillus mucosae and Enterococcus casseliflavous.

Conclusion: Although the LAB in the rumen may be linked to acidosis and some metabolic disorders, they can be used as a probiotic for young ruminants such as calves or lambs. Also LAB can be used as a source of inoculums for ensiling some plants. Their ability to produce bateriocins is currently investigating which implies their potential uses as natural antibiotic for manipulating of rumen fermentation.

Keywords: Ruminant, Lactobacillus, Enterococcus, RFLP, BLAST

P346: **The survey on interleukin-10 -592 polymorphism in Iranian infected patients with HBV**

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Background and Aim: Infection with hepatitis B virus (HBV) may result in a number of different clinical outcomes. There are strong evidences in HBV infection that host genetic factors play a major role in determining the outcome of infection. Cytokines have an important role in immune defense. Single nucleotide polymorphisms (SNPs) in the promoter region of the interleukin 10 (IL-10) genes have been reported to play a role in determining of HBV infection outcome. The aim of present study was to investigate the association between HBV infection and -592 polymorphism in the promoter region of the IL-10 gene in Iranian population.

Methods: A total of 200 cases including 100 hepatitis B patients and 100 healthy controls were enrolled in this study. Samples were tested for HBsAg by ELISA and HBV-DNA by PCR procedure. Total genomic DNA from peripheral blood leukocytes was extracted by the salting out procedure. Then one biallelic (-592A/C) polymorphism in the IL-10 gene promoter was analyzed by allele specific amplification (ASA) PCR.

Results: No significant difference was found in frequency of genotypes of IL-10 gene promoter region at position -592 between controls and patients, but frequency of CC genotype was higher in the hepatitis B patients than that in the controls (12%vs16%).

Conclusion: It appears that -592 polymorphism of IL-10 gene are not associated with HBV infection outcome in our study.

Keywords: single nucleotide polymorphisms (SNPs); interleukin 10 (IL-10); hepatitis B virus; allele specific amplification (ASA) PCR.

P347: **In silico DNA Fingerpriting of Geobacter Species with Digested Fragments on PFGE**

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Background and Aim: Geobacter species have several potential applications in Nano biotechnology for the creation of microbial Nanowires, and known to have unusual electron transfer and environmental restorative capabilities, and also several industrial uses like Natural battery and interference in Bioremediation processes .in this research

Methods: we have used Bioinformatics tools for analysis of DNA fingerprinting pattern of Geobacter species with loading digested DNA fragment on a simulated Pulsed Field Gel Electrophoresis(PFGE) in 1.2% agarose with Molecular Weight Markers (Lambda ladder),we have used type IIb and type IIs restriction enzymes such as AsiSI,RgaI,SgfI ,MssIand PmeI

Results: we have constructed dendrograms based on UPGMA algorithm with distance matrices based on Dice method and displayd band patterns.

Conclusion: there are some differences between distinict restriction enzymes and gel profile patterns,it will be noatable matter for detection phylogenetc relation ships and biotechnological applications.

Keywords: PFGE,Dendrogram,Nanowires

P348: **Synthesis of TiO2 nanopowder by sol-gel method and investigation of its antibacterial activity on Escherichia coli PTCC 1395**

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Background and Aim: Increasing of drug resistance and so the extension of Escherichia coli infections have drawn the attention of scientific communities. So, finding of new antimicrobial compositions has a considerable importance. TiO2 nanopowders have large surface areas, thus increases the levels of their contact with microbes.

Methods: In this study, TiO2 nanopowders were synthesized by using sol-gel method and their physico-chemical properties were characterized by the scanning electron microscopy (SEM), X-ray diffraction (XRD), and Fourier transform infrared spectroscopy (FTIR). Photocatalytic properties of the prepared TiO2 nanopowders were investigated by inactivation of E. coli PTCC 1395 under irradiation of UV-B lamp.

Results: The results showed that the TiO2 nanopowders at 0.1 molar concentration had only anatase phase while at 0.3 and 1 molar concentrations both anatase and rutile phases were observed. The size of nanopowders was estimated 28.70, 31.05 and 32.94 nm for 0.1, 0.3 and 1 molar concentrations, respectively. The sharp peaks in FTIR spectrum determined the purity of TiO2 nanopowders. The prepared TiO2 nanopowders have significant antibacterial effect on E. coli PTCC 1395 at concentration of 1mg/ml. Raising the molarity from 0.1 to 1 decreased the viable cells concentration of E. coli PTCC 1395 from 88.89% to 8.33% during 3 hours.

Conclusion: It is recommended that our investigation can be followed in vivo and use of this nano powders for therapeutic aims in the future.

Keywords: TiO2 nanopowder, E. coli PTCC 1395, Sol-gel, photocatalytic

P349: **E-test antibiotic susceptibility of Escherichia coli strains isolated from hospital acquired infections of Imam Khomeini hospital, Ilam, Iran**

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Background and Aim: Since, drug resistance of common bacteria cause hospital infections and also regards to disk diffusion method as an available antibiotic susceptibility test, obtain the Minimum Inhibitory Concentration (MIC) of antibiotics by E-test method in compared with disk diffusion method is very functional. Therefore, the aim of this study is to determine the pattern of antibiotic resistance in clinical isolates of Escherichia coli that isolated from Imam Khomeini hospital in Ilam by E-test method.

Methods: In this cross-sectional study, 30 samples were collected from medical centers and hospitals in Ilam. After detection, bacteria were transferred to TSB and incubated for 3 to 4 hours at 37 ° C to achiebe 0.5 McFarland concentrations. Then, microbial suspension cultured on the surface of Mueller Hinton agar and E-test strips were placed and incubated for 18 to 24 h at 37 ° C. Finally, the MIC of each strip was determined by E-test method according to standard tables.

Results: The results shown that susceptibility of bacteria to amoxicillin was 17/5%, and for other antibiotics were: Ticarcillin 37/3%, Meropenem 100%, Ceftazidime 93/4%, Cefepime, Nitrofurantoin and Piperacillin 90/1%, Ceftriaxone, Tetracycline, and Trimethoprim Sulfamethoxazole 83/3%, Ceftriaxone 80%, Ciprofloxacin 76/6% and Gentamicin and Tobramycin 73/3 %.

Conclusion: Our funding indicated that the highest sensitivity was to Meropenem, Ceftazidime, Cefepime Nitrofurantoin, Piperacillin and minimum sensitivity was to Amoxicillin and Ticarcillin. Hence, according to antibiotic resistance increasing in hospitals, there is necessary to a considering to drug prescribing and reasonable usage of antibiotics based on infection control committee guideline.

Keywords: Escherichia coli, antimicrobial susceptibility, E-test.

P350: **Prevalence of microorganism in related to urinary tract infection in Iran; A Met-analysis**

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Background and Aim: Urinary tract infection (UTI) is one of the most prevalent infection diseases. The prevalence of this infection in Iranian studied was varied. Therefore, provide an overall estimate of the prevalence of this infection is very import. The aim of current study is to evaluate the prevalence of UTI and determine the most microorganisms of UTI in Iran by using met analysis method.

Methods: Searching done by specific keywords on Databases such as: SID, Magiran, Iranmedex, Sciencedirect, Pubmed and Google Scholar. 40 papers were selected between 1995-2012 years, according to approaches to enrolled in study. All papers after qualitative control by using random effect model enrolled to Meta analysis. Heterogeneity between studied was assessed by I-Square index, and then data were analyzed by using R software (Version 2.11.1) and STATA software (version 10).

Results: Among 40 selected papers, 11 papers were eligible to enroll Meta analysis. Overall, 66,448 individual were studied. The prevalence rate of UTI in Iran was 13.3% (CI95%; 7.8-17.8). E.coli by 62.1% (CI95%; 55.4-68.9) was the most prevalent microorganism and Staphylococcus aureus by 4.8% (CI95%; 3.6-6.0) was the lowest isolated microorganisms.

Conclusion: In order to reduce the prevalence of UTI, Health education, prescribe appropriate antibiotics based on urine culture, and avoided more taking antibiotics and self medication recommended.

Keywords: microorganisms, UTI, Meta analysis, Iran

P351: **Genotypic Analysis of Class 1 & 2 Integron Genes in Haemophilus infuenzae Isolated from Clinical Isolates of Iran**

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Background and Aim: Haemophilus influenza is a gram negative rod-shaped bacterium that is classified the unencapsulated and the encapsulated. H. influenza type b consist of 90% of all of them that show itself in half of cases like meningitis. High levels of multidrug resistance are normally associated with mobile genetic elements that encode specific resistance genes. Integrons are natural tools for bacterial evolution and innovation. Integron is a significant genetic system involved in spreading antibiotic multiresistance. Its gene sequences contribute to spreading antimicrobial resistance alleles by lateral gene transfer of gene cassettes. Gene cassettes can be transferred along with mobile genetic elements. A gene cassette is a modular DNA sequence encoding one or more genes for a single biochemical function. Genomic islands have also been regarded as segments of DNA acquired by horizontal gene transfer. The large exogenous gene fragments often contain genes which relate to the survival of the organism under adverse conditions.

Methods: Twenty clinical isolates were collected from different patients of Milad hospital by Dr Rahbar's group. Antibogram tests were conducted about Amoxicillin (A), Ciprofloxacin (CIP), Ceftriaxone (CRO), Clindamycin (CD), Azithromycin (ATH), Chloramphenicol (C), Tetracycline (TE), Trimethoprim (TM) and Sulfamethoxazol (SXT). The PCR reactions were performed by using universal primers attributed to Int1 and Int2 about Integrons. Also amplifications of integrase gene attributed to Genomic Islands were investigated by designing the specific primers.

Results: As total 20 number of H. influenza 20 isolates (100%) were resistant to Clindamycin, Chloramphenicol and Tetracycline, 95% to Amoxicillin, 50% to Ceftriaxone, 45% to Ciprofloxacin and 5% to Azithromycin. Also 20 isolates (100%) were sensitive to Trimethoprim and Sulfamethoxazol. In spite of the positive results of phenotypically, class 1 and 2 integrons were detected in no isolates; but integrase gene attributed to Genomic Islands was identified in 12 isolates.

Conclusion: Sometimes gene cassettes don’t express on integrons. Also some isolates that have resistance phenotype but they don’t have its gene cassette on the integron. In these cases, other factors and genetic elements, except integrons, are responsible for antibiotic resistance. The results of the present study confirm this point. Pay attention to the past researches, there is high prevalence of class 1 integrons and trimethoprim/sulfamethoxazole resistance. In this study all of isolates were sensitive to Trimethoprim/Sulfamethoxazol. It can be a good reason for lack of class 1 integrons justification. Also, it can be a confirmer for geographical heterogeneity, because isolates of this study have been native and they have different genetic base. Findings of this study, confirm Genomic Islands as an efficient strategy in order to gain of antibiotic resistance genes. Variations between phenotypical & genotypical results become justification because of different pathway for resistance engendering.

Keywords: Haemophilus influenzae, Integron, Gene Cassettes, Genomic Islands.

P352: **The survival rate of coccoid forms of H. pylori in water samples**

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Background and Aim: Helicobacter pylori is an important cause of human chronic gastro mucus's infections, peptic ulcers and stomach cancers. Once the bacterium is placed in the water it changes in to a viable but non-culturable form, which considers as an important factor to spread out the infection through the water. The aim of this study is evaluate the PCR test and culture for recognize coccoid forms.

Methods: This experimental study was performed on 10 strains isolated from clinical specimens. Isolates were added to water at 3 different temperatures and were incubated at 1 and 2 months. Each time, the samples were cultured on the Brucella blood Agar culture, DNAs were extracted and PCR test was performed based on ureC gene.

Results: No positive results in non of the samples was observed in culturing method in recognition of the cocooid forms in 4°C during first month but some positive result was detected in 22°C, 37°C equal to 10%, 20% and during the second mounth no positive result was detected. The result of PCR method in the 4°C, 22°C, 37°C were included 10%, 30%, 40% for the first month and 0%, 20%, 30% for the second month, respectively.

Conclusion: In this study, it was proved that the non-culturable cocoid forms of Helicobacter pylori are detected by culturing independent methods such as PCR which are highly sensitive.

Keywords: Helicobacter pylori, coccoid, ureC gene, PCR, Culture

P353: **Chitinase production by Actinobacillus sp.**

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Background and Aim: Chitin is the second most abundant polymer in nature after cellulose. It is found in crustacean, fungi, protozoa and insects. Chitin degradation is a key step in cycling of nutrients in the environment. The first step in chitin digestion is hydrolysis of β-1,4 linkages by chitinase. Chitinase are extracellular inducible enzymes that are found in variety of organism. Demands for chitinase increased due to its increased usage in many fields such as controlling environment pollution. Commercial interest is to find inexpensive and reliable source of stable chitinase. The present report was an attempt to selected and identified bacteria which able to produce chitinase enzyme.

Methods: Various samples of crab shell waste and seafood industrial waste were collected from south of Iran. Screening of chitinolytic microorganisms was performed on chitin agar plates. Those strains which higher clear zone in shorter time were selected and incubated into liquid chitin medium for measurement activity enzyme. The reducing sugar released was measurement at 540nm. Those strains which showed the highest chitinase activity were identified using molecular marker and submitted with specific gene bank database.

Results: By comparison of gene sequence, the selected strain was identified as Actinobacillus. In additional showed special species can be used as a suitable source for chitinase production.

Conclusion: The study reflects the potential of special species to produce chitinase for environmental application, biotechnological applications such as N-acetyl glucosamine production and protoplast formation and agricultural application.

Keywords: Chitinase, Actinobacillus, Colloidal chitin

P354: **Sequencing and Molecular characterization of Phlebotomus papatasi, principle vector of zoonotic cutaneous leishmaniasis by targeting elongation factor_1α and cytochrome b genes in Khuzestan province.**

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Background and Aim: Zoonotic cutaneous Leishmaniasis (ZCL) is a tropical and subtropical disease caused by protozoan parasites. Sandflies are vectors of ZCL in human and reservoirs host animals in rural areas of Khuzestan province, Iran. Some of Phlebotominae sandflies are indistinguishable via morphological characters but molecular methods have a valuable diagnostic role in various sandflies' genus detection. The main objective of this study is to accurately identify the main vector of ZCL using amplifying and typing of targeting genes to discerning different species of vector-borne- disease leishmania sandflies

Methods: In the peak of sandflies' activity seasons, samples were collected using sticky paper, aspirator and CDC miniature light traps from different locations of Khuzestan province, Iran. After dissection, sandflies were slide-mounted on berlese medium and primary identification was carried out based on the morphological keys of the head and abdomen terminalia. DNAs were extracted from samples. Subsequently, Elongation factor_1α and Cytochrome b genes were amplified using standard PCR, sequenced, typed and analyzed. Insilico methods was performed to confirm the final results

Results: Out of 3000 samples 150 different sandfly species were selected, DNAs of all individuals were amplified and screened by PCR. Two samples had enough DNA to sequence the fragments of targeting genes. These samples were contained main and possible vectors of leishmaniasis in Khozestan.

Conclusion:: Using morphological characters in some female sandfly species are indistinguishable. By employing elongation factor_1α and Cytochrome b genes not only different sandfly species were identified but also different haplotypes of each sandfly species were found.

Keywords: Molecular typing, Sandflies , Cytochrome b, Elongation factor_1α, Khuzestan.

P355: **Cloning and expression of human Keratinocyte Growth Factor in E. coli in order to produce a recombinant drug**

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Background and Aim: Keratinocyte growth factor (KGF) is a member of the fibroblast growth factor (FGF) family that induces proliferation and differentiation in a wide variety of epithelial tissues. KGF plays an important role in protection, repair of various types of epithelial cells and re-epithelialization of wounds. Therefore, in patients with hematologic malignancies receiving high-doses of chemotherapy and radiotherapy, treatment with KGF decreases incidence and duration of severe oral mucositis. The aim of this study was to express the recombinant form of human keratinocyte growth factor in E. coli.

Methods: KGF gene was amplified by PCR and cloned into the expression vector pET28a (+). Recombinant vectors were transformed into E. coli BL21 (DE3) as expression host and expression of desired protein was induced by IPTG. The expression was evaluated at RNA and protein levels by RT-PCR and SDS-PAGE analyses and identity of the expressed protein was confirmed through western blotting.

Results: Cloning procedure was confirmed by PCR and restriction digestion. RT-PCR and SDS-PAGE represented expression of KGF in E. coli. The optimized expression was achieved 16 hours after induction with 0.3mM IPTG at 37°C in LB broth containing kanamycin. The 18 kDa protein was confirmed in western blotting with anti His6 antibodies.

Conclusion: The result of the present study indicated that E. coli expression system is suitable for overexpression of recombinant human KGF and the expressed protein can be considered as a home made product.

Keywords: Cloning, recombinant protein, keratinocyte growth factor.

P356: **Seroprevalence of Cytomegalovirus IgM antibody in Hormozgan juvenile by Enzyme- linked immunosorbent assay**

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Background and Aim: . The transfusion transmitted CMV is possibly dangerous complication of transfusion therapy in immunocompromised patients. The aim of this study is to determine the seroepidemiology of Cytomegalovirus IgM antibody in Hormozgan juvenile.

Methods:: In this study the presence of Cytomegalovirus antibody was also evaluated in 200 serum samples from healthy adolescnce Hormozgan province by Elisa test in virology department of Tehran university in order to diagnosis CMV antibody

Results:: Of the sera tested, % 9 were found to be Cytomegalovirus IgM positive.The frequency seropoitivity reveald no significant differeence between boy and girl. .regarding to high rate of CMV infection, it is expected transplant patients in Iran face less clinical problems for matching transplant donors and recipient.

Conclusion:: These findings have important clinical application, because the detection CMV positive sera may reduce the risk for transmission of CMV in blood and therapy also decrease the risk for CMV induced complication .In the transplant recipient acute infections is very important and due to existence of new suitable antiviral drug for treatment ,a rapid and sensitive methods for diagnosis of infection is necessary

Keywords: Cytomegalovirus , Enzyme- linked immunosorbent assay (Elisa),Hormozgan

P357: **The study of parasites infection (protozoan and worm) in clients at central laboratory in Amol from 1390 to 1392**

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Background and Aim: Intestinal parasites have a worldwide distribution and are considered as a major public health and economic problem throughout the world. It is estimated that you cannot find anywhere in the globe that it has been not infected by different types of parasites diseases. Therefore, the current study was designed to determine frequency of parasitic infection in clients at central laboratory in Amol from 1390- 1392

Methods: Cross sectional survey was conducted and stool samples were examined among those clients who had visited central laboratory in Amol from 1390 to 1392. Collectively,18720 stool sampels were examined and parasitic detection was confirmed by formalin athyle acetate,and concentration method. Scotch glue technique has been used for detection of Enterobius Vermicularis

Results: We recruited17820 subjects (96079 males, 9113 femals) to participate in this survey. Consequently1183(6/31)were parasites infection, stool test positive for protozoan was found in468 (39/56)and for worm was found 715(60/43). The frequency of intestinal parasite infection was found to be high among Trichostrongylus, Entamoeba coli

Conclusion: To sum up, in this study, the main prevalent pathogenic parasites were Trichostrongylus,Entamoeba coli And the less prevalent pathogenic parasites were Strongyloides

Keywords: Amol ,parasites infection

P358: **COMPARISON OF CULTURE AND MOLECULAR METHODS FOR THE IDENTIFICATION OF NOCARDIA SPP. FROM BRONCHOALVEOLAR LAVAGE (BAL) SPECIMEN**

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Background and Aim: Pulmonary Nocardiosis is a severe infection with a high morbidity and mortality that mainly affects immunocompromised patients. For the diagnosis of pulmonary nocardiosis, samples including from sputum, tracheal aspiration or bronchoalveolar lavage. The morbidity and mortality brought about by nocardiosis can be substantially reduced through prompt diagnosis and management. Conventional identification of Nocardia species in routine medical laboratories which is based on phenotypic methods is often laborious a time-consuming. The need for new methods allowing identification of Nocardia is crucial. The objective of this study was to compare of culture and molecular methodsand develop a rapid and new method of identifying clinically relevant Nocardia species.

Methods: In this study, 116 patients with advanced symptomatic pulmonary infection were studied in the course of a 8 month period. The specimens were cultured in BHI agar, BHI agar with kanamycin(BHIAK),BHI agar with parafin(BHIAP) and sabouraud dextrose agar (SDA), and then incubated in 37oC. Nocardial DNA was extracted with handel methods. In the PCR(polymerase chain reaction)method, NG1 and NG2 primers were used to amplify a Nocardia genus- specific 598-bp fragment of 16SrRNA. Sequencing results were analyzed by Mega 5 software and using Nocardia Spp., sequences from Gene Bank.

Results: As the important finding of the present research,7 samples(%6.03) were positive with PCR, and 5 samples (%4.3) were positive with culture method. All samples with positive cultures also were positive PCR. Using molecular and culture methods characterized that 1case of species was belong to N.otitidiscaviarumand 6 cases of species were belong to N. cyriacigeorgica.

Conclusion: Rapid and accurate diagnosis of species Nocardia is essential for treatment severe infections and prevention of cerebral abscess. This study showed that PCR have high sensitivity and accuracy in the detection of Nocardia spp. compared with culture.Considering the rapidity, precision, high sensitivity and specificity of molecular techniques, in the future, it is recommended that we use of this technique with other methods in detecting phenotypic Nocardia spp. in laboratories, medical centers and research.

Keywords: Nocardia spp. , Culture, PCR , Sequencing.

P359: **Evaluation of serum antibody titers of E.coli K99 antigen in laboratory animal**

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Background and Aim: Escherichia coli(ETEC) is one of the most important causes of post weaning diarrhea in calves. This diarrhea is responsible for economic losses due to mortality, morbidity, decreased growth rate and cost of medication.The aim of this work was evaluation and Immune responses against enterotoxigenic K99-fimbriated Escherichia coli in rat model.

Methods: Bacteria were cultured in appropriate media broth so collected by washing with phosphate-buffered saline (PBS), pH 7.2, and centrifugation at 3.000xg for (15 min at 4oC). To inactivate the bacteria Formaldehyde0.4% was added to the suspension to a final concentration of 0.5%. One volume of solution was added to one volume of the suspension as solution of aluminum hydroxide adjuvant. after inactivation twenty-four rats were immunized intramuscularly by inactivated Escherichia coli K99 solution. Rats were injected with 0.5 ml of solution twice and three rats were un-immunized as controls. Measure anti-K99 antibodies were characterized by indirect enzyme-linked immunosorbent(ELISA) tests.

Results: Rates from treatment group of this experiment showed higher anti-k99 serum antibody titer compared to the control group ($p<0.05$).

Conclusion: Further investigation is needed to improve the antigen for preventing calf diarrheal disease.

Keywords: Escherichia coli, k99, diarrhea,calves,Elisa

P360: **Antibiotic resistance patterns of Serratia strains isolated from blood cultures of the patients referred to Namazi Hospital in 2010, Shiraz, Iran**

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Background and Aim: In the recent years the problems associated with hospital infections caused by Serratia strains have become increasingly evident. The ability of this opportunistic pathogen to acquire resistance to a broad range of antibiotics has made effective therapy more difficult. Several recent investigations have dealt with the problem of antibiotic resistance in Serratia. This study was undertaken to evaluate the antibiogram patterns of the clinical Serratia strains isolated from the blood samples of the patients.

Methods: The blood sample of the patients, taken in bactec bottles were cultured and investigated for any causative infectious agents. Identification and final confirmation of the isolated organisms were done using biochemical- microbial tests and laboratory diagnostic Microgene kit. Antimicrobial resistance of the strains to 12 antibiotics was evaluated using the standard method of the Kirby- Bauer agar disk diffusion test.

Results: Totally, 22 Serratia strains were isolated from the blood specimens of the patients during March 2010 to March 2011. The data showed that; 57.8%, 55%, 43.7%, 36.8%, 33.3%, 31.8%, 25%, 22.7% and 15.78% of the Serratia strains were resistant to ceftriaxone, tobramycin, cefotaxime, cefepime, amikacin, gentamicin, ceftizoxime, ciprofloxacin and ceftazidime, respectively. No resistance to meropenem, imipenem and piperacillin/tazobactam was seen among all the isolates under the study.

Conclusion: Comparing the present data with the previously data achieved in other studies, show an obvious increase in antibiotic resistancy in clinical isolates of Serratia. However, the effects of carbapenems and piperacillin/tazobactam in vitro on these bacteria are considerable. These results mandate the local monitoring of resistance and its consideration in empirical therapy of Serratia infections.

Keywords: Antibiotic resistance pattern,Serratia strains, blood culture

P361: **Establish of loop – mediated isothermal amplification (LAMP) technique for rapid diagnosis of Fusarium solani in culture and clinical samples**

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Background and Aim: Fusarium solani is a common soil saprophyte and plant pathogen which is also frequently been reported as etiologic agent of opportunistic superficial and disseminated mycoses, in humans and animals. The most virulent Fusarium sp. Is Fusarium solani and mortality rate due to this kind of disseminated Fusariosis is almost 100%. However, by suitable methods for rapid and accurate diagnosis, the irreparable consequences of this infection , would be avoidable. The purpose of this study is designation and optimization of loop – mediated isothermal amplification (LAMP) technique for rapid diagnosis of Fusarium solani in clinical samples.

Methods: The DNA was extracted from standard strain of Fusarium solani, PTCC NO.5284, and a set of six specific LAMP primers were designed for TEF-1α as a target gene. The test was optimized and its specificity and sensitivity were evaluated. Afterward, the test was used for detection of standard DNA and for 10 other culture of Fusarium solani from plant and animal sources. After confirmation the result of optimized test and evaluation of its specificity and sensitivity , it was used directly on clinical samples. These samples were include of 45 DNA specimens, extracted from serum of Aids patients and 100 DNA extracted from keratitis ulcers. The total of samples were tested by PCR method as a gold standard and the results of two test were compared by chi-squared test.

Results: All of the cultures were identified by the optimized LAMP test and the obtained results from standard strain were verified. The specificity of LAMP test were 100% for detection of Fusarium solani and its sensitivity was one copy of genome in culture and clinical samples. The test was able to diagnose organism in one hour and in compare with PCR test ,which can diagnose it in 3 hours , it is much more faster . By using this test among 45 Samples belong to aids patients, 9 samples were positive for Fusarium solani and among 100 keratitis samples , 2 cases were positive . The same results were obtained by PCR. Comparing the results of these two methods by chi-squared test, show that both of them are equal to detection Fusarium solani.

Conclusion: Because of accuracy , rapidity and its cost effecting , the LAMP technique can be a better alternative for PCR in clinical laboratories.

Keywords: LAMP, Fusarium solani, Clinical samples, Culture

P362: **Isolation and identification of Leishmania parasites in suspected patients of Cutaneous Leishmaniasis by targeting ITS-rDNA and Microsatellite genes, Shush city, Northern Khuzestan (2012-2013s)**

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Background and Aim: Zoonotic Cutaneous Leishmaniasis (ZCL) is a protozoan disease which is caused by Leishmania major. ZCL is considered as a prominent public health disorder in some specific provinces of Iran particularly in Northern Khuzestan. In Iran-Iraq war, Shush city was one of the significant foci of ZCL. L. major is the principle causative agent of ZCL in Iran. In addition, Phlebotomus papatasi has a crucial role as a main vector and Tatera indica is the most important reservoir of ZCL in this region. Species determination of Leishmania is essential to perform a useful program for controlling, surveillance and monitoring of ZCL. This study was done to identify the Leishmania parasites using microscopic and molecular methods in suspected patients of Cutaneous Leishmaniasis by targeting ITS-rDNA and Microsatellite genes, Shush city, Northern Khuzestan, Iran.

Methods: 65 slides prepared from suspected patients' lesions of ZCL, in Northern Khuzestan, Iran during 2012-13, samples were stained and examined under a light microscope. In order to diagnose of mix parasites, DNA of parasites were extracted directly from prepared slides and ITS-rDNA and Microsatellite genes were amplified. Positive samples digested with BsuRI restriction enzyme according to RFLP method and consequently the parasite was identified. Microsatellite markers were used for detection of weak bands from ITS-rDNA gene. Afterwards, molecular methods and sequencing was performed to verification of the ITS-rDNA gene. It is noting that insilico method was used for confirmation of RFLP results.

Results: 50 out of 65 and 52 out of 65 samples were detected as Leishmania positive using microscopic observation and molecular techniques respectively. All 50 positive samples digested with BsuRI endonuclease and identified as Leishmania major. In order to reconfirmation, 4 samples of L. major were sequenced and confirmed by using of CLC DNA workbench software analysis.

Conclusion: Comparison of obtained sequences of present investigation with GeneBank sequences in a case of homology was confirmed L.major in human from Shush city, Northern Khuzestan. Other species of Leishmania were not identified in this study. These findings should be considered to improve the disease control programs which can be led to increase the rate of public health in Khuzestan province

Keywords: Leishmania major, ITS-rDNA, Microsatellite, Shush, Iran

P363: **Cloning and purification of BauA, an outermembrane receptor involved in iron uptake of Acinetobacter baumannii**

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Background and Aim: Introduction: Acinetobacter baumannii is a gram negative bacillus classified as one of nosocomial infection causing bacteria. This bacterium can cause a range of infections such as bacteremia, pneumonia, meningitis, urinary tract infection, and wound infection. Multiple-drug resistance of the bacteria has made the treatment of the infection very difficult. Its ability to survive and resist to a wide range of antimicrobial agents has made the researchers seek for alternate diagnostic and treatment tools. In this study we aim to target a surface protein of this bacterium and develop an antibody based assay for diagnosis and therapeutic use of Acinetobacter baumannii infection.

Methods: Material and Methods: Target antigen: After bioinformatics studies BauA surface siderophore receptor was chosen. Epitope mapping studies resulted in selection of plug region and β-barrel section of the protein. Gene cloning and protein expression: Two specific primers were designed for isolation and amplification of the selected epitopes from purified genome of Acinetobacter baumannii ATCC 19606T. Selected DNA segment was then cloned into pET28a expression vector. The cloning process was analyzed by plasmid digestion and PCR-RFLP. Recombinant constructions were transferred into E. coli BL21 (DE3). Expression was induced with 1mM IPTG at 37o C for 4 hours. Expression was analyzed on 9% SDS-PAGE. Recombinant protein was purified using Ni-NTA chromatography and analyzed on 9% SDS-PAGE and western blotting using anti-His antibodies.

Results: Results: Expression and purification: Induction with 1mM IPTG resulted in high expression of recombinant BauA protein. The expression resulted in accumulation of protein in cytoplasmic inclusion bodies. To dissolve the inclusion bodies, cytoplasmic contents were incubated for 16 hours in a buffer containing 100mM NaH2Po4, 10mM Tris base and 8M Urea (pH 8). SDS-PAGE analysis resulted in a thick protein band of 68 kDa. Ni-NTA chromatography purification resulted in a single band of 68 kDa designated to recombinant BauA protein. The expression was confirmed with western blotting using HRP-conjugated anti-His antibodies. Same 68 kDa band was observed in a nitrocellulose paper.

Conclusion: Discussion: Acinetobacter baumannii is a serious threat in compromised patients. The bacterium is also responsible for infections including septicemia, meningitis and more recently necrotizing fasciitis. Emergence of multiple-drug resistant strains of Acinetobacter baumannii has complicated treatment of the infections specially in patient with weaker immune system under intensive care. Bacterial iron acquisition is a well-studied process playing a significant role in bacterial survival and infection. BauA is an outermembrane receptor involved in the transport of acinetobactin. Acinetobactin is involved in acquisition of iron from bacterial surrounding and plays significant role in bacterial viability. Thus, neutralizing bacterial ability to absorb iron can eliminate the bacterial infection. Production of anti-BauA antibodies can either be used as a therapeutic tool or as a diagnostic approach for early diagnostic of the bacterium infection. Multiple-drug resistant strains of Acinetobacter baumannii encouraged researches to seek alternative approaches to treat the bacterial infections. Previous studies have successfully used antibodies as an alternative to antibiotic therapy of bacterial infections.

Keywords: Keywords: Acinetobacter baumannii, BauA protein, Iron uptake receptor, Outer membrane protein.

P364: **The effect of mesenchymal stem cells on Neutrophil phagocytic function in rat**

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Background and Aim: Mesenchymal stem cells (MSCs) ,a population of adult stem cells , are a promising source for therapeutic applications. MSC also showed a potent immunomodulatory mature immune cells. This study was done to investigat the effect of Rat MSCs on the performance neutrophils phagocytosis.

Methods: MSCs was isolated from Rat (6-8 weeks) femoral bone marrow and cultured in DMEM. The mature cells were incubated with neutrophils isolated from peripheral blood of Rat at 37 ° C for 1 h. Neutrophil phagocytic function was messured by the rate of yeast (Candida albicans) phagocytosis and Nitro Blue Ttrazolium reduction (NBT) test. Data were analyzed by SPSS software,t-test at a significance level of $p<0.05$.

Results: The rate of phagocytosis in neutrophils treated with MSCs was decreased compared to control group;but this effect was not significant $p<0.05$.The respiratory burst in neutrophils treated with MSCs, significantly decreased compared to the control group $p<0.05$.

Conclusion: Neutrophils are one of the first defence cells recruited at tissue microenvironment due to infection and inflammation.On the other hand, in this study the interaction between MSC and neutrophils were shown. Therefore, such interaction maybe considered in the treatment plan with MSCs.

Keywords: Mesenchymal stem cells, Neutrophils, phagocytosis

P365: **Gaussian model and calculation of pollutant Bioaerosols Maximum concentration at critical conditions.**

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1Higher Education of rab Rashid

Background and Aim: As an air pollutant is transported from a source to a potential receptor, the pollutant disperses in to the surrounding air and finally it arrives at a much lower concentration than it was on leaving the source. Strict Crisis is a conditions in which sudden and uncontrollable events occur and threat human life and environment. environmental regulations worldwide resulted in an ever growing concern about the validity and reliability of pollutant dispersion models.

Methods: The current study is descriptive-analytic English and Persian resources and Reports & Data of different s experiment are used in this study and data was analyzed.

Results: The present work is a try to evaluate the applicability of GPM which modeling the pollutant dispersion from point source at ground level or above. Effect of important factors like wind velocity, temperature, emission release rate, propagation height and … on pollutant concentration and their diffusion are calculated by this model and the results are given in detail.

Conclusion: So, for prediction of how pollutants disperse from different sources, mathematical models should develop and simulate the effect of atmospheric process and many other factors.

Keywords: Dispersion, Pollutant Concentration, Gaussian Model, Sensitivity, Atmospheric Condition

P366: **Long-term Preservation of Aerobic and Anaerobic Probiotics in IBRC**

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Background and Aim: Probiotics are live microorganisms especially bacterial strains that may affect a health benefit on the host. Lactic acid bacteria (LAB) and bifidobacteria are the most common used as probiotics, but certain yeasts and bacilli may also be used. Probiotics are commonly consumed as part of fermented foods with specially added active live cultures, such as in yogurt, soy yogurt, or as dietary supplements. Therefore, preservation of probiotics for industrial and clinical purposes has an important effect as Iranian Biological Resource Centre (IBRC) is studying of methods to preserve them effectively.

Methods: In this study, three aerobic (IBRC-M-10666 Lactobacillus sakei subsp. Sakei, IBRC-M-10692 Lactococcus lactis subsp. Lactis and IBRC-M-10711 Lactobacillus casei) and anaerobic (IBRC-M-10730 Lactobacillus delbrueckii subsp. bulgaricus, IBRC-M-10754 Lactobacillus rhamnosus and IBRC-M-10755 Lactobacillus reuteri) LAB were selected to be preserved by two methods included cryopreservation and freeze drying using different protectants. Cryo-protectants for storage in -80°C included 20% (v/v) glycerol and 25% skim milk and a simple disaccharide (sucrose). Combination of this suspension had an effective role in recovery of cryo-preserved LAB in comparison to control without cryo-protectants.

Results: Result indicated after one year storage in -80°C, aerobic and anaerobic LAB were viable but aerobic ones showed uncountable growth in 10-7 and 10-8 dilutions. No differences were detected between aerobic and anaerobic freeze dried LAB. Furthermore, we examined the use of sucrose and sodium glutamate instead of trehalose as lyo-protectants for these strains. All freeze dried strains (aerobic and anaerobic) showed viability after 6 months but 40% of them had uncountable growth in 10-7 and 10-8 dilutions that confirmed suitable effects of sucrose and sodium glutamate that is comparable with trehalose.

Conclusion: Significantly, disaccharide such as sucrose used in this study decreases the damage developed during preservation and it helps recovery of long-term preserved LAB. Despite the further studies determined freeze drying is the best method for long-term preservation of probiotics especially LAB, our study showed cryo-preservation with a combination of different protectants is a remarkable method for these bacteria.

Keywords: Probiotics, Lactic Acid Bacteria, Cryo-preservation. Freeze Drying

P367: **Application of sensitive papers in microbiology laboratories to quality control disinfectant sprays, and contamination**

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Background and Aim: Control of contamination in Microbiology Laboratory, is an important fact. For this purpose, it is necessary not only to prevent microbial suspension bursting, but also antiseptics for disinfecting surfaces are desired. Therefore, it is necessary to measure the effectiveness of disinfectants and its performance will be. The procedure for spraying should be checked. The performance of employees with the infected material should be controlled, too. This papera are one of the tools that are sensitive to water base disinfectants and microbial suspensions. After contact with water base reagents, the color of the paper changes. These papers which are used in agriculture can be prepared in different sizes. The surface of these papers are covered with a non-ionized indicator and can be used in critical areas.They will change color after contact with disinfectants and microbial suspentions, so the particles that are inadvertently thrown will be traceable. If the papers are used to control disinfecting surfaces, the number of droplets per unit area, shows how spray acts. Changed sensitive papaers can be kept as a record of each assay. Indicator is usually an acid or base which its color is different in the molecular and ionized state.

Conclusion: Control of contamination has an important role in safety of the personel and environment, especially in large labs. .As microbial suspensions can change the color of these papers, so any contamination can be traceable.In addition, the efficiency of spreading disinfectant sprays can be evaluated.

Keywords: biosafety-quality control-sensitive papers

P368: **Quantification of bacterial cells by using rapid and simple MTT[3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay and spectrofluorometery methods**

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Background and Aim: Microbial counts are useful in basic science studies and can be used to determine the number of bacteria in physiological and biochemical studies. The food samples are tested for determining the number of pathogenic microbial agents in the food industry. Clinical laboratories monitor the growth rate of bacteria from patients to determine their antimicrobial sensitivity. Sometimes to study the efficacy of an antiseptic agent or the growth rate of bacteria, the number of viable bacteria is important. Although the old method of counting bacterial cultures is used, but the traditional methods are time consuming and may work poorly with slow growing or viable, but non-culturable organisms. Hence, we need to have a culture-independent, fast and accurate methods for counting.

Methods: MTT assay was carried out on the previously determined the CFU of E.coli(origami) washed 3times with PBS. By plotting a OD curve, The minimum CFU that can be detectable by MTT assay was determined. Using fluorescent properties of DNA binding redsafe dye and after optimizing the concentration of color, using emission curve we can determine minimum CFU. In this study in order to increase accessibility of bacteria DNA to redsafe, bacteria suspension was lysed by boiling and sensitivity of spectroflurometery surveyed in this modified method.

Results: In this study, the sensitivity of MTT assay to determine CFU was appointed 2×107. The sensitivity of spectrofluorometery method was determined 8×106 CFU. With enhance accessibility of DNA by boiling, the sensitivity of spectrofluorometery reduced to 5×106 CFU.

Conclusion: The spectrofluorometery method is more sensitive than MTT assay. In some cases where the number of bacteria in samples are low, boiling and using spectrofluorometery method can be accurate estimation of the number of the bacteria. . If all bacteria in sample were dead, determination of CFU by using traditional culturing methods is impossible, but using spectrofluorometery for determination of CFU because of redsafe bounding to DNA, make it possible to determination of CFU. The quenching phenomenon in fluorescent redsafe could interfere with results. MTT is more stable over several hours after the test and it dose not decrease the degree of OD. when bacterial growth is well, MTT assay is easier than spectrofluorometery. Both MTT and specrofluorometery methods are not time-consuming and do not need bacteria culture.

Keywords: CFU, MTT assay, Spectofluorometery

P369: **Isolation of endophytic bacteria from Glycin sp. and evaluation of their antimicrobial effect on plant pathogenic bacteria**

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Background and Aim: Endophytic bacteria are bacteria that live in the plants tissues without any apparent damage to them. Nowadays, many scientist evaluate the plants for isolation and characterization of endophytic bacteria, because some of them have antifungal and bacterial activity and some use as fertilizer which could help the growth of plants.

Methods: In the present study endophytic bacteria were isolated from stems and leaves of soybean plants using serial dilutions (10-1- 10-8). Then the isolates were evaluated for ability to produce anti microbial metabolites against two bacterial plant pathogens: Xantomonas campestris(PTCC 1473) and Erwinia caratovora(PTCC1675) based on parallel streak and well diffusion agar methods .The bacterial strains with ability to produce anti microbial metabolites were identified by molecular method. In addition, during the evaluation of antimicrobial effect the growth curve were evaluated for the same strains.

Results:: In general, 39 bacterial endophytes were isolated from stems and leaves of the soybean plant. Out of all 20 were belonging to the stems and 19 were isolated from the leaves. Furthermore 12 strains showed the antimicrobial effect but two strains had more effect on plant bacterial pathogens which they were belong to the genus of Pseudomonas. In addition the results obtained from the growth curves of the bacterial isolates indicated that most of the microorganisms produced their compounds at the first 24 hours which is continued up to 72 hrs in some isolates.

Conclusion: The present study showed that soybean plant containing endophytic bacteria have ability to produce antimicrobial effect against plant pathogens and the produced compounds can be used in future research.

Keywords: Endophytic bacteria, Glycine sp. Pseudomonas

P370: **The Comparative Study of Antimutation and Anticarcinogen Effectes of Green Tea Extract On Mutagenicity and Carcinicity Effectes of Natural and Flavored Tobacco by Ames Test and Salmonella TA100**

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Background and Aim: It should be considered that the main cause of global prevalance of tobacco consumption developing since 1990 have been producing mix flavored tobacco and today for desired fragrance of essence which have been attained more than natural tobacco this tobacco has been popular,Disadvantages of this material consumption it should be proven by scientific documents. Scientific goals of this research are: The study of anti mutagenicity and anti carcinogenicity effectes of green tea extract on mutagenicity and carcinogenicity effectes of natural tobacco and Flavored tobacco by Ames Test and Salmonella Typhimurium TA100, and if it prove that tobacco essence lead to cancer and mutation consumption of this chemical material will be reduced as possible, and if it prove that green tea has anti cancer and anti mutagen effect its consumption in society specially among people who smoke kalian will be increased (operational goals).It haven't been performed so far in Iran.

Methods: In this study we took the advantage of flavored tobacco and liquid extract of leaf of green tea and natural tobacco and performed the determination test of mutagen and antimutagen potential based on proposed method of Professor Ames et al.using mutant strain of Salmonella Typhimurium TA100 and well known carcinogenic material(Sodium Azid).Also by adding microsome of rat liver (S9) the cancer effect of type of tobacco extracts and anticancer effect of green tea extract was proved.In each test, a positive and anegative control containing sodium azid and distilled water, respectively were studied.Ech test was simultaneously done for three times and the percentage of inhibitory was determined according to (1-T/M)×100.

Results: Based on results of statistical data analyse about natural and flavored tobacco all kinds of tobacco were tested and led to cancer by 95% confidence,and flavored tobacco leads to mutation and cancer more than natural tobacco. It also proved green tea has the inhibitory percentage equal to 47-60%.

Conclusion: By this research it proved scientifically that in despite of public beliefe tobacco smoking through water can't refine cancering chemical matters and flavored tobacco leads to mutation and cancer more than natural tobacco. Also by this research it proved green tea reducing cancer and mutation effects induced by tobacco.Therefore this is a new aspect of social health and public information enhancement.

Keywords: Ames Test,Green Tea,Natural and flavored tobacco, Salmonella Typhimurium TA100

P371: **New mutations in plasmodium vivax dhfr and dhps genes in Hormozgan province, Iran**

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Background and Aim: Molecular markers are useful approach for recognition of mutation in P. vivax. The aim of this study was genetic analysis of the dihydrofolate reductase-thymidylate synthase and Dihydropteroate Synthetase Genes in P. vivax in Hormozgan province for recognition of mutation at codons 33, 57, 58, 117, 173 and 382, 383, 512,553, 585 related to antifolate drug resistance

Methods: From April 2007 through January 2008, a total of 118 blood samples were collected from P. vivax malaria patients in two endemic areas of Hormozgan province, south of Iran. PCR-RFLP and sequencing methods were used.

Results: After sequencing analysis, One novel mutation were found at codons 421 in pvdhps gene in three isolated and no new mutant were found in pvdhfr gene.

Conclusion: In spite of this fact that the antifolate drugs are not prescribed for P. vivax malaria, observed mutant alleles in pvdhfr and pvdhps gene of P. vivax is probably due to expose of P.vivax with fansidar drug.

Keywords: Dihydrofolate reductase-Thymidylate synthase gene, Dihidropteroate synthetase gene, Plasmodium vivax, PCR-RFLP, Sequencing, Hormozgan province

P372: **Susceptibility of gram positive and gram negative bacteria to nanosilver colloidal formulation**

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2- Assistant Professor in Horticulture science
3- Student of Microbiology
4- graduated from veterinary laboratory science

Background and Aim: The bactericidal properties of silver nanoparticles against a broad spectrum of bacteria were obtained. In this study bactericidal effect of one type of nanosilver formulation against some important clinical bacteria was evaluated.

Methods: Characteristics of the bacterial growth inhibition of one colloidal formulation of silver noparticles with the average size of 4-5 nm were quantified against Pseudomonas aeroginosa, Escherichia coli, Klebsiella pneumoniae, Bacillus cereus, Staphylococcus aureus, Bacillus subtilis and Listeria monocytogenes, based on disk diffusion tests. The nanosilver disks were prepared using 4000, 2000, 1000, 500, 250, 125 and 62.5 ppm of silver nanomateriales in concentration. Ampicilline and penicillin were used as control.

Results: All gram positive and gram negative bacteria used in this study, had sensitivity to nanosilver particles. The highest antibacterial effect was observed at 4000ppm against Bacillus subtilis. No significant difference was obtained in antibacterial effectivity of concentrations of 4000, 2000 and 1000 ppm against used bacteria. Rates of the highest and lowest inhibition zone at 4000 ppm were 38 mm (Bacillus subtilis) and 10 mm (Pseudomonas aeroginosa) respectively. Susceptibility to nanosilver at 250, 125 and 62.5ppm was not observed in used bacteria.

Conclusion: Silver nano materials could be a good choice as disinfection and antibacterial agent specially in multidrug resistances. Using effective concentrations of the agent against bacteria will help to reduce the consumption of nanosilver . Species-specific sensitivity to nanosilver materials was determined but rates of susceptibility to nanosilver could not differentiate gram positive and gram negative bacteria.

Keywords: Nanosilver, Antibacterial effect, Bacillus subtilis, Pseudomonas aeroginosa

P373: **Antibacterial effect of two types of nanosilver colloidal formulations on Escherichia coli isolates from infectious disease of human and poultry**

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1- assistant professor in Microbiology

Background and Aim: Because of the emergence of multiple antimicrobial resistance in human society and animal farms, specially poultry with high relationship rate to human health in our country, development of new potentiated agents is needed.

Methods: The antibacterial effect of two types of silver particles colloidal formulations with 4 and 20 nanometers was investigated on 30 isolates of E. Coli from human urine and 40 isolates from diseased poultry based on inhibition zone diameter in disk diffusion tests. The disks with concentrations of 20, 40, 80, 400 and 4000 ppm of nanosilver particle was prepared in this study.

Results: The antibacterial effect of nanosilver increased with slaking of nanoparticle size. At 4000 ppm concentration, The average of inhibition zone using 4 nanometer particles against human isolates were 57% higher than the zone using 20 nanometer particles. In Poultry isolates zone of inhibition about 4nm and 20nm paticles were 140mm and 95mm. The highest diameter inhibition zone of human isolates was 21 mm and it was evaluated in two isolates of E.Coli at 4000 ppm concentration of 4 nanoscale. No sensitivity was observed in more than 45% of all isolates based on both types of colloidal formulations in 80 ppm.

Conclusion: Results showed that applied bacterial isolates primarily were affected by particle sizes and in the second, by concentration levels of nanosilver. Totally, it can be concluded that particle size and concentration level have correlation with inhibition of bacterial growth. These materials could be an effective agent for disinfecting of waste water but for their comprehensive applications, more investigation is necessary.

Keywords: Nanosilver Particles, Escherichia coli, Disinfection, Antibacterial effect

P374: **The Evaluation of The Anti-Quorum Sensing Property of Whitetop And Dill For The First Time In The World**

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Background and Aim: Many bacteria utilize quorum sensing mechanism in order to coordinate their vital functions such as survival, motility, the production of biofilm, pathogenicity factors etc. Interfering with the complicated cell-to-cell communication system paralyses bacteria to perform their indigenous functions as in the potential of the pathogenicity. The study is considered to be the first report of its kind in Iran and the world and was carried out for the purpose evaluating the effects of two distinguished plant extracts, whitetop and dill, on the interference of the natural and sophisticated bacterial quorum sensing of Chromobacterium violaceum CV026.

Methods: Whitetop and dill plant species were collected from the surrounding agricultural areas and commercial fields of Urmia City. The collected plants were extracted using three organic solvents, 96% ethanol, n-hexane and methanol. The Antimicrobial susceptibility and anti-quorum sensing bioassays were then performed to find out their bactericidal property and depletion of violacein, respectively. What's more, the assays regarding pathogenicity suppression using anti-quorum sensing activity and acyl homoserine lactone induction through Pectobacterium carotovorum subsp. carotovorum strain were carried out.

Results: The results of the present research disclosed that both of the plant extracts possess meaningful anti-QS activities; however, the proportion of the activity in whitetop was fewer than dill. Furthermore, the aforementioned plant extracts had bactericidal activity in which whitetop had more proportion in comparison to dill.

Conclusion: According to the amount of violacein inhibition in CV026 due to the anti-quorum sensing activity of whitetop and dill extracts, application of the plant extracts can be considered as an appropriate approach for controlling bacterial pathogens without developing resistance.

Keywords: AHL, CV026, DMSO, Inhibition, Quorum Sensing

P375: **Evaluation of S.griseofuscus antagonistic activity against isolated bacteria from infected urethaltracts**

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Background and Aim: The Streptomyces sp .are the most valuable prokaryotic cells in economical and biotechnological manner. They produce about half of bioactive secondary metabolites.includeing antibiotics ,antitumor agents and enzymes.

Methods: The six bacterial species ,Acinetobacter sp and identified E.coli ,K.pneumoniae ,S.marcescens, P.aeroginosa,and P.mirabilis were isolated from urine patients according to differential and selective tests. It was used spot inoculums and pour plate procedures for assessing of antagonistic activity of S. griseofuscus strain against clinical isolates.The submerge method was used for production of antimicrobial materials using ISP2 medium.Finally ,The agar well diffusion method was used for final screening for antibiotic susceptibility.

Results: S.griseofuscus Indicated had antimicrobial activity against E.coli and S.marcescens using spot inoculums. Also,it had antimicrobial activity against on E.coli and Acinetobacter using pour plate method. This activity had been showed on S.marcescens, P.aeroginosa P.mirabilis, Acinetobacter sp, E.coli and K.pneumoniae using agar well diffusion method and ISP2 medium was used for Producing Bioactive Substances .produced bioactive components by S. griseofuscus were identified using GC/MS.The separated components of bioactive compounds including 3-hexene-2-one‹) 1,1-Diethoxyethane (Diethyl acetal ‹1-Propoxy-2-propano ‹2,6-dimethyl-2,5Heptadien-4-one‹2-Heptanol ‹ Eicosane and 10-methyl elcosane.

Conclusion: .The demonstrated antibacterial effect of This strain suggests that it may be one of new medicinal resources for antimicrobial agents.

Keywords: Streptomyces, Antimicrobial activity,Agar well diffusion method

P376: EXPERIMENTAL DESIGN AND DESIRABILITY FUNCTION APPROACH FOR EXPLORATION OF SILVER NANOPARTICLES STABILITY AND POTENCY ON DIFFERENT IN VITRO CULTURING MEDIA

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Background and Aim: One of the key points of the biological properties of engineered nanoparticles is related to their nano-meter characteristics. Their size increment following by growth/aggregation/agglomeration processes may strongly influenced on their biological potency or even deactivated them. The investigations on the life-cycle of the engineered AgNPs mentioned that, the processes may govern the toxicity of AgNPs in any circumstances are the results of the combined influences of nature, concentration and the exposure path of the nanoparticle as well as the environment, biology and the functional ecology of the involved organism. Furthermore, it reported that the environmental water content plays an important role in the stability of AgNPs, the rate of the aggregation or ion release. The proper hydrolic conductivity is essential for the mobility and encounter of AgNPs. The risk assessment on the fate of AgNPs requires documentations, which are not currently available. But enough is however known to be certain that risks must be investigated. In order to understand the impact of applying AgNPs to the environment, the primary step is to study their stability, potency and transportation on the culturing media. The aim of this study is to evaluate the impact of the different microbiological and biotechnological in vitro cultivation parameters including their content, cultivation temperature and time, shaking and the exposure to light on the stability and final antibacterial characteristics of silver nanoparticles.

Methods: The stability of the colloidal AgNPs at different concentrations (0, 10, 30, 50 and 70 µg/mL), temperature (20, 25, 34, 40 and 45 °C) shaking and light/dark conditions for times of 1, 6, 13, 20 and 24 h on seven different media including; Gamborg's B-5 (B5), Murashige & Skoog Medium (MS), Chu salt mixture (N6), Nutrient (N), Mueller-hinton broth (MHB), Youshida (Y) and the homemade Carbon source/Vitamin mixture (CV), over 160 designed experiments were studied. The stability and exposure features of the treated AgNPs were studied based on their Ultra Violet-Visible spectroscopy (UV-Vis) absorption spectrum and the Dynamic light scattering (DLS) for their size and size distribution homogeneity and the Transmission Electron Microscopy (TEM) for their morphological variations. Their instability and changes based on the mentioned variables, responses and a desirable function were modeled using the Design Expert 6.0.1 software. Moreover the antibacterial properties of the resulted AgNPs were studied using the Minimum Inhibition Concentration (MIC), Minimum Bactericidal Concentration (MBC) and the zone of inhibition methods against staphylococcus aureus and Escherichia coli.

Results: The results and the model suggest that the increment in the temperature and duration of cultivation in the exposure of light and shaking lead to the severely decrease on the AgNPs stability and biological potency. In addition, the more complex culturing media; the more rapid loss of stability. Moreover, the additives including different carbon source or vitamin accelerate the AgNPs instability rate.

Conclusion: The models are shown the stability, function and potency of AgNPs and provide the information for researchers using them.

Keywords: Chemicals & Bioassays Data & Software

P377: **Anti-rabbit polyclonal antibody production, purification and the development of an ELISA**

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Background and Aim: Antibodies are used in many diagnostic and experimental procedures.The primary goal in experimental polyclonal antibody production is to obtain sufficient volumes of high titer and high-affinity antibodies economically. Anti-rabbit immunoglobulins and its conjugate with horse radish peroxidase (HRP) is used to diagnose rabbit infectious diseases by ELISA or Western blotting tests.

Methods: In this study, the production, purification and HRP conjugation of polyclonal IgG against rabbit immunoglobulins in rats were carried out. Five 6-month-old Male Wistar rats were immunized by rabbit immunoglobulins in combination with Freund's adjuvant. Purified antibody using ion-exchange chromatography was labeled to HRP. Direct ELISA was used to determine the optimum titer and cross reactivity of HRP conjugated IgG.

Results: The purity of various IgG preparations was about 98%. The optimum dilution of prepared HRP conjugated IgG was 1: 25600. This conjugated IgG has no cross reactivity with guinea pig and hamster immunoglobulins at optimized dilution.

Conclusion: This study showed that ion-exchange chromatography could be an appropriate method for purification of IgG antibodies.

Keywords: Rabbit, Production, Purification, ELISA

P378: **Molecular characterization of Tatera indica the main reservoir hosts of Zoonotic Cutaneous Leishmaniasis in Khuzestan province of Iran.**

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Background and Aim: Zoonotic Cutaneous Leishmaniasis is one of the protruding public health issue and an endemic parasitic, vector-borne disease existing in most regions of Iran including Khuzestan province. Tatera indica is the main reservoir hosts of Leishmania parasites. There is not enough morphological information of Tatera indica. No research and investigation which has been implemented on molecular features in Iran and even in the world has been done yet. This study, it is the first time that the number of this gerbil's species characterized molecularly. The Mitochondrial Cytochrome b (Cyt b) gene from these rodents, which is a maternally-inherited gene marker, were used for characterization and sequencing for genus and species verification via molecular methods

Methods: In our investigation, rod and wooden live catch tarps with cucumber as bait, were applied for capturing rodents. DNA extraction has been performed by phenol chloroform and ISH-Horovize methods. Moreover, Cyt b gene amplified, sequenced and analyzed using molecular software.

Results: 10 Taetra indica rodents were distinguished among captured gerbils based on diagnostic and morphological keys and molecular characters. DNAs of all collected samples from different regions were extracted and amplified from the mentioned gene (Cyt b). Sequenced samples were shown that this rodent have a unique sequence (haplotype)

Conclusion:: Out of 624 extended nucleotide of Cyt b gene were applied for phylogenetic analyses, whole specimens of this species were placed in a single network. In simultaneous investigations in this endemic area after detection of L.major infection in these rodents, we can definitely incriminate Tatera indica as the main reservoir host of ZCL in Khuzestan province, Iran.

Keywords: Leishmaniasis, Cytochtochrom b gene, Tatera indica, molecular typing, Iran

P379: **Evaluation of Biological Modification Effects on Shearing Stress of Clayey Sands .**

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Background and Aim: Materials such as Portland cement, gypsum, sodium, epoxy, etc. are currently used to stabilize soils and improve their mechanical parameters. Most of these materials are generally toxic and can damage the environment. So, a great number of scholars, researches, and activists pay special attention to biological modification of soils using bacterial strains to achieve desired objectives . Microbial carbonate precipitation is one of these bio-processes.

Methods: Sporosarcina pasteurii PTCC 1645 (DSM 33) was the bacterial strain used in this study. Square soil specimens (6 × 6 cm and 2 cm in height) were prepared by mixing bacterial suspension, nutrient solution, and silica sand having a quite uniform grain size. After 28 days of curing time, the samples were removed from the molds and assessed in direct shear machine.

Results: The results revealed a significant increase in the final shearing stress of biologically-treated soil samples based on t-test.

Conclusion: Biological soil stabilization has a remarkable effect on soil strength parameters that increase ultimate shear stress of the soil equal to and sometimes over 2 to 3-fold increase. According to the experimental results, the amount of nutrients consumed by bacteria and biomass volume were directly influential to the test results.

Keywords: Biological soil modification, carbonate precipitation, shearing stress, silica soil

P380: **Antimicrobial study of Dorema auchri on some normal flora and pathogen Intestinal microorganisms**

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Background and Aim: Due to emergence of resistance to antibiotics amongst microorganisms, investigation for novel antimicrobial agents have always been one of the major preoccupation of medical society, traditional medicine system have played an important role during human evolution and development.Today a number of plants medicine are studied for their medicinal activities around the world .Amongst the Several plants medicine, that are used world wide as a medicine, Dorema auchri is yet another potent plant medicine which, has not been extensively studied for the medicinal uses in comparison with other plants medicine Dorema auchri has a long history of use as a sore and food additine in Yasuj. However, not much scientific work has been done Dorema auchri therefore the present investigation aims to study the antimicrobial properties of Dorema auchri on some normal flora and pathogen Intestinal microorganisms

Methods: serval experiments were carried out in orther to evaluate the antimicrobial effect of Dorema auchri.After collection and preparation of hydroalcoholic extract of Dorema auchri, this extract were studied against microorganism. The determination of minimal inhibitory Concentration (MIC) and minimum lethal concentration were evaluted for this extract.The antimicrobial potent of Dorema auchri extract with commercial antimicrobial were compared.

Results: the finding of this study was showed that in l0 mg/ml and 20 mg/ml Concentration, all bacteria were resistant to Dorema auchri, in 40 mg/ml maximum ZOI was 12mm in Escherichiea coli and in concentration: 80 mg/ml ZOI was 18, 8, 6 & 6mm in E.coli,shigella , salmonella & serratia respectively.The acceptable MIC and MLC were 40 mg/ml & 80 mg/ml in Epidermis and E.coli respectively.

Conclusion: Our data clearly indicate that the extract show eqivalent compatibility with standard Antibiotics. This will be natural compounds neutraliticals used for curing diseases rather using synthetics or semi-synthetic antibiotics.

Keywords: Dorema auchri, Intestinal Microorganism

P381: **Antimicrobial Activity of OAK BARK on Staph Areus & Staph Epidermidis**

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Background and Aim: Due to emergence of resistant to antibiotics amongst microorganisms' investigation for novel antimicrobial agents has always been one of the major preoccupations of medical society. Plant medicine system have played an important role during human evolution and development because they have low side effect and economical in comparative with another chemotherapeutic agents. Amongst the several plants medicine Oak Bark is a plant medicine that has not been studied as antimicrobial agent. Ancient people used this plant to treat wound infection such as: Malignant wound, Mycobacterium Leprosy, Gastric ulcer and duodenal ulcer. As a noticed aim of this study was antimicrobial

Methods: In this study we prepared hydroalchoholic extraction from Acorn of Oak Bark and measurement zone of inhibition on Muller Hinton Agar by well assay method that contaminated with staph areus and staph epidermidis then we measurement MIC and MLC of this extraction on Staph Areus & Staph Epidermidis

Results: This study showed that MIC 32 mg% on Staph Areus & Staph Epidermidis and MLC was 64 & 32 mg% on Staph Areus & Staph Epidermidis respetly. This extract showed good zone of inhibition (20 & 22 mm) on Staph Areus & Staph Epidermidis that in comparative with industrial antibiotics this extraction showed good result.

Conclusion: Hydroalchoholic extraction from Acorn of Oak Bark showed good result on Staph Areus and stagh Epidermidis in comprative with standard Antibiotics then We hope in futher with more work on this plant medicine we can use this extract as antibiotic.

Keywords: ,Oak Bark (Acorn), Antimicrobial Activity

P382: **Simultaneous detection of Leishmania parasite and Wolbachia bacteria in main vector of Zoonotic Cutaneous Leishmaniasis in Abarkouh district, Yazd**

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Background and Aim: Leishmania major is the main causative agent of Zoonotic Cutaneous Leishmaniasis (ZCL) and the main vector of ZCL in Iran among other old world countries is Phlebotomus papatasi (Phlebotominae family). Wolbachia bacteria are gram negative Riketssia like bacteria's which can be found in reproductive system of most Arthropods. They cause cytoplasmic incompatibility (CI), Parthenogenesis induction (PI), feminization and male killing between different strains and transfers horizontally. These reproduction malfunctions in hosts has elective results for the bacteria.

Methods: Abarkouh district of Yazd province Iran is a focus of ZCL in Iran, but there has been no record of a study on the disease, vectors and the infection rate of the vectors with Wolbachia in the area. This is a simultaneous study on the infection rate of Wolbachia bacteria and Leishmania parasites in the vectors of ZCL in Abarkouh district. The Sandfly samples were collected from Abarkouh during the peak of sandfly activity using sticky papers, CDC miniature light tarps and aspirators. Sandflies were dissected and mounted, and their genus and spices were identified. DNA was extracted from their thorax and abdomen. For Leishmania detection PCR was performed targeting ITS-rDNA, microsatellite DNA and minicircle kDNA. And as for detection of Wolbachia infections in the samples PCR was performed targeting wsp gene.

Results: More than 3000 sandflies were collected from Abarkouh and 540 of them were dissected and identified. 150 of them were female P.papatasi which 25 of them were infected with Leishmania after PCR. Using RFLP and also Sequencing the ITS-rDNA gene of positive samples definitely confirmed L.major infection in P.papatasi. Of 33 different spices of Phlebotomus sandflies studied for Wolbachia infection 6 were positive. All the Wolbachia found in the area was confirmed to be type A Wolbachia. Based on results of this study we can incriminate L.major as the main causative agent and P.papatasi as the main vector of ZCL in Abarkouh district of Yazd Province.

Conclusion: The low rate of parasites found in female sandflies of the area could be caused by Wolbachia infections in those samples. The low amount of parasite in sandflies can be detected using microsatellite gene, but with long genes as ITS-rDNA or even kDNA we need more parasites DNA in samples for detection. It is possible that because of the Wolbachia infection in sandflies, Leishmania parasites could not been reproduced or they could not finish their life cycle. But based on this study we can definitely conclude that Wolbachia bacteria and Leishmania parasites can exist in main vectors of ZCL. But its effects and reactions are yet to be discovered. For confirmation of this theory more studies are required.

Keywords: Leishmaniasis, Phlebotomus papatasi, Wolbachia bacteria, Abarkouh, PCR

P383: **Superficial and cutaneous mycoses in Mashhad, Iran**

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Background and Aim: Superficial and cutaneous mycoses are infections limited to the outermost layers of the skin, the nails and hair, and the mucous membranes. The principal infections in this group are the dermatophytosis, infections with malassezia spp and superficial forms of candidiasis. These infections are the most common infectious diseases in the world and are among the most commonly diagnosed skin diseases in Iran and considered as the major public health problems in many parts of the world. The prevalence of the diseases varies in different geographical locations. The aim of this study was to determine the prevalence of superficial and cutaneous mycosis and site of the infections in Mashhad, Iran.

Methods: This study was conducted for a period of 4 year during April 2009 to march 2013. A total of 1520 patients clinically suspected to have superficial and cutaneous mycoses who were referred to the Medical Mycology Laboratory of Quaem Hospital, Mashhad University of Medical Sciences, were studied. Samples were taken from different sites of patient's body include; trunk, scalp, face, hand, foot, mucous membranes, groin, toe and finger nails. In the cases of tinea capitis and tinea barbae, hair samples were taken in addition to scraping from lesions. All collected specimens were analyzed by direct microscopy. Microscopic examination was carried out using 15% KOH for skin samples, 20% KOH for nail clippings and lactophenol for hairs.

Results: From 1520 patients (874 female and 646 male) suspected of having fungal infections 688 cases (45.3%) suffered from these infections; among them 408 (59.3%) cases were female and 280 (40.7%) cases were male, ranged in age from 1 to 80 years. Of 688 patients with superficial and cutaneous mycosis, pityrosporosis (danddruf) with 533 cases (77.5%) was the most common type of fungal infection followed by dermatophytosis (12.5%), tinea versicolor (6.4%) and candidiasis (3.6%). Tinea corporis and candidal onychomycosis were the most common types of dermatophytosis and candidiasis respectively.

Conclusion: superficial and cutaneous fungal infections are still the most common fungal diseases in Mashhad. Promotion of public health care and self hygiene and knowing the frequency of these infections may play an important role to control of the infections.

Keywords: Superficial mycosis, cutaneous mycosis, dermatophytosis, candidiasis, Mashhad

P384: **Evaluation of AJc-F nanocomplex efficacy to prompt hepatitis B vaccine immunogenicity**

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Background and Aim: Hepatitis B vaccine doesn't induce proper immune responses in a significant percent of the population due to alum insufficiency; thus designing and synthesizing an efficient adjuvant in order to achieve an appropriate preventive vaccine is essential. In this study we evaluated the adjuvant effect of Ajc-F nano complex which was synthesized to improve immunogenicity of hepatitis B vaccine and compared it to ferrous sulfate.

Methods: Female Balb/c mice were divided to several groups and injected 3 times with hepatitis B vaccine. The proliferative response of the splenocytes was evaluated using bromodeoxyuridine assay and interleukin-4 (IL-4), interferon-gamma (IFN-gamma) levels and total specific antibodies were examined by the ELISA method. Data analysis was performed by SPSS software version 18.

Results: AJc-F nano complex significantly increased total antibody and cytokine levels compared to ferrous sulfate and control groups. Ferrous sulfate severely decreased total antibody and IL-4 levels compared to control group. Both compounds increased splenocyte proliferation and IFN-gamma levels compared to control group.

Conclusion: The results show that AJc-F nano complex could efficiently elevate humoral and cellular immunity in hepatitis B vaccine.

Keywords: Adjuvant, Nanotechnology, vaccine, Hepatitis B, Iron

P385: **Determination of Aspergillus isolates DON(Deoxy nivalenol) production pattern , A definitive toxigenicity a mongest 24 different species of Iran**

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Background and Aim: Mycotoxins are important chemotoxicant since they are mainly produced in large feed stock subtraets Where fungis producing these toxins find optimum conditions for growth and development and they may become the cause of occurrence of some disease in humans and animals. Thus , their presence in foods and animals feed is a subject of global or national importance. Among them , Aspergillus has particular importance, In present study we will study the production of DON toxin in cell extract of Aspergillus isolated ecogeneous in Northern Iran

Methods: Firstly, sampling, culture and isolation was performed in Guilan and Mazandaran provinces. After recognition of the species, using ELISA and Ridascreen kit®,we quantitively analyze DON produced by available species

Results: Some of species has toxin production but amount of toxin production in a large number of isolates was small

Conclusion: In identified species the maximom produced DON is drived by species A.sp VI with concentration 34.93 ppb and minimom produced DON observe in the A.carbonarius with concentration 1.03 ppb

Keywords: Aspergillus,DON,Species,Iran

P386: **Development of a multiplex PCR for detection of common causative agent of respiratory tract infections**

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Background and Aim: Pneumonia is caused by a range of bacteria and viruses. Community Acquired Pneumonia is a leading cause of disease in different populations. The number of hospital admissions of patients was significantly and Imposes heavy costs to the health system .The rate of hospitalization due to community acquired pneumonia, is between 22 to 50% that, in 4% of cases are fatal. The disease mortality is more children than any other disease. Each year, causes approximately 4 million deaths worldwide in children. In developing countries, the figure is about 2 million children less than 5 years, per years. The incidence of bacterial pneumonia can be divided into two categories: hospital-acquired pneumonia and community-acquired pneumonia. Staphylococcus aureus and Klebsiella pneumonia are the most common cause of hospital-acquired pneumonia, and Streptococcus pneumonia and mycobacterium tuberculosis are Cause of community-acquired pneumonia During the past decade, improvements were made to identify the causes of pneumonia. Specific PCR and Multiplex PCR (conventional and time-real) is a very fast and reliable method to detect respiratory pathogen in clinical samples and recently have developed.

Methods: Multiplex PCR is a rapid, accurate molecular method for the simultaneous detection of two or more specific genes in a reaction. In this study, multiplex PCR method for simultaneous detection in the genome-specific sequences leading causes of pneumonia (Streptococcus pneumonia, mycobacterium tuberculosis, Staphylococcus aurous and Klebsiella pneumonia) are used. In all organisms, there are genes or sequences that are specific for the same organism (such as house-keeping genes). Specific genes that we chose in this study are: ply (pneumolysin) for Streptococcus pneumonia, nuc (thermonuclease) for the staphylococcus aureus, gyrA (gyrase A) for Klebsiella pneumoniae and pncA gene for Mycobacterium tuberculosis, also the 16s ribosomal gene was used as a positive control. After set-up procedure and observing specific bands, the bands were extracted from the gel and for final approval sequencing was done

Results: Our results showed that the detection of Streptococcus pneumoniae, Staphylococcus aureus, Klebsiella pneumoniae and Mycobacterium tuberculosis in a Multiplex PCR reaction is possible

Conclusion: In fact, this method can be used for direct and simultaneous detection of these agents in the respiratory specimens.

Keywords: multiplex PCR ،respiratory tract infections, Streptococcus pneumonia, Staphylococcus aureus, Klebsiella pneumonia, Mycobacterium tuberculosis

P387: **Screening and isolation of xylanase bacteria and identification of selected strains based on molecular marker**

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Background and Aim: xylanolytic enzymes from microorganisms have important role in biotechnology. This enzyme use in industrial processes such as food, feed, pulp and paper industries, biobleaching, biofuels production and in the production of pharmacological. In this study we aimed to isolate and identify bacteria showing high xylanolytic activity.

Methods: Different samples of agriculture wastes were collected in Iran. Xylnolytic bacteria were isolated by beech wood xylan culture and selected strains identification base on molecular marker 16S rDNA. Initial screening 30 bacteria showed high clear Zone on XC-agar plates were selected for determine extracellular and intracellular xylanase enzyme activity. The final 18 bacteria showed high xylanase activity were selected and all isolates identified base on 16S rDNA molecular marker. Xylanase activity of isolates was measured using di-nitro salicylic acid (DNS) method. The nucleotide sequence 16S rDNA of the tested isolate was compiled and compared with sequences in NCBI using a BLAST program.

Results: This study maximum xylanase production was observed by special spices of Sphingobacterium and the partial 16S rDNA gene sequence of this strain was deposited in the GenBank nucleotide sequence database under the accession number.

Conclusion: Xylanase producing bacteria were isolated and optimized in order to utilize for industrial strains development for biologic processes instead chemical.

Keywords: Xylanase, Sphingobacterium, Screening, 16S rDNA

P388: **candidiasis vulvovaginit**

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Background and Aim: Vulvovaginal candidiasis is a common disease among women specially in reproductive ages that approximately 75% of them experience at least one episode of VVC during their lives

Methods: . In this study sterile cotton swabs were used for sampling from vaginal region from 120 women with and without symptom .samples inoculated on Sabouraud dextrose agar then to distinguish candida species inoculated on human serum, corn meal agar+ tween 80, chrom agar then unknown species distinguish with Rapid yeast kit. Prevalence of candida among 120 women enrolled was found to be 45 samples. The NCCLS protocol was followed and 45 yeast isolates were tested for their susceptibilities to fluconazole by the broth microdillution method.

Results: . A total of 45 Candida strain were isolated from VVC patients including 26 (57.7%) Candida albicans,10 (22.2%) Candida glabrata,5 (11.1%) Candida krusei, 2 (4.4%) Candida parapsilosis and 1 (2.2%) Candida tropicalis and 1 (2.2%) Candida kefyer. Isolates of Candida species were 33.3% Resistant, 37.7% sensitive and 28.8% susceptible dose dependent to fluconazole. In this study Candida albicans was the most species but 42% of Candida species were non-albicans

Conclusion: this result shows increasing non albicans species.Resistance to Fluconazole in these isolates is high and it is better to use from another antifungal drugs.

Keywords: vulvovaginitis , Fluconazole, Candida

P389: **Molecular detection of Leishmania major parasites in Rodents, the reservoir hosts of Zoonotic Cutaneous Leishmaniasis in Khouzestan province**

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Background and Aim: Leishmania major is the main causative agent of Zoonotic Cutaneous Leishmaniasis (ZCL) in Khouzestan province and Tatera indica species has been incriminated as a main reservoir hosts in this region. The only assay used to firmly identify Leishmania parasites in rodents is molecular method. Whereas ordinary laboratory methods have not yet been accurate enough to detect of Leishmania, therefore, the Leishmania major parasites have been typed by targeting Microsatellite gene

Methods:: Tatera indica rodents were captured using wooden and wirily live traps then were identified by diagnostic keys and molecular tools. Smears of each ear were prepared from serous of scratched ears. DNA was extracted by Genet Bio kit. In addition, Microsatellite gene was amplified by Nested PCR and measuring band size. Thereby, Leishmania species has been surely approved by their sequences.

Results: Out of 33 rodents captured from Khozestan province, 11 were accurately identified as Tatera indica. 6 of these rodents (54.54%) were detected to be infected with Leishmania parasites using Microsatellite gene PCR and then 3 of those (3/6 =50%) were confirmed using ITS-rDNA gene PCR. Then the positive samples were sequenced and using molecular software helped us to definitely identify L.major in Tatera indica rodents as the main agent of ZCL.

Conclusion: In this investigation, Tatera indica rodents were discriminated from other gerbils. L. major was diagnosed after DNA extracting and amplifying of relevant gene in this rodent. Using nucleotide sequencing of the L. major gene by SequencherTm 4.1.1 software, the sequences were edited, aligned and compared with the registered microsatellite gene of L. major in GenBank in a case of similarity and homology. All sequences (Forward & Reverse) were made to a single network (contig) and analyzed systematically with MEGA and PAUP software which were employed for phylogenetic analysis. The isolated sample of Leishmania major from this rodent (Tatera indica) was contained one common and/or general haplotypes and one new unique haplotypes which are being reported for the first time.

Keywords: Leishmania major, ITS1-5.8s-ITS2 gene, Microsatellite gene, Tatera indica, Khouzestan province.

P390: **The role of probiotic of Lactobacillus casei and Lactobacillus paracasei isolated from common carp intestine in rat**

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Background and Aim: Lactobacillus species are genetically diverse groups of Lactic Acid Bacteria (LAB) that have been introduced as probiotics, because of some characteristics such as their anti-tumor properties, helping the intestinal flora balance, production of antibiotics, stimulation of host immune response, decrease serum cholesterol, etc. The goal of this study was to evaluate the role of probiotic of Lactobacillus casei and Lactobacillus paracasei isolated from common carp intestine in rat.

Methods: The intestinal contents of 115 common carp captured from the natural resources of West Azerbaijan province in Iran were examined for LAB. After isolation, the identification of Lactobacilli was done according to traditional and molecular bacteriological tests. Subsequently, a suspension of each bacterium was prepared separately. Twenty four male rats (175± 25 g) randomly divided into two experimental groups each with triplicate. One group selected as control and only received normal saline. another group fed with L. casei and L. paracasei in combination (5×108 CFU/ml). The animals were fed with 1.5 ml for 4 weeks and trial was continued for two weeks later without receiving any additives. In this study biometry at the day of 0 and 30, biochemical parameters (ALAT, ASAT, ALPH, Cholesterol, Triglyceride, Total protein, Glucose, Creatinine, Uric acid) were assayed at the day of 30 and 45. Data were analyzed using one-way ANOVA by SPSS software.

Results: Results showed the rat serum profile changed independently each other. At day 30, the level of ASAT, ALPH, Cholesterol, Triglyceride, Glucose, Creatinine and Uric acid had not any statistical differences with the control. The level of ALAT and total protein statistically ($p<0.05$) was higher than the control group. At the day of 45, statistical ($p<0.05$) differences were not seen between groups. growth parameter in rat gavaged with lactobacillus had no significant increase the level of 0.05.

Conclusion: It should be concluded that the administration of L. casei and L. paracasei isolated from common carp intestine could increase the rat serum protein level and improve immune system, also could improve the rat growth parameter besides increasing the level of serum ALAT. For obtaining the best results more study must be done.

Keywords: probiotic, lactobacillus casei, lactobacillus paracasei, growth, blood biochemical profile, rat

P391: **Study on synergistic effect of raftilose with Lactobacillus casei and Lactobacillus paracasei in rat**

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Background and Aim: The goal of this study was to evaluate the synergistic effect of raftilose with Lactobacillus casei and Lactobacillus paracasei in rat

Methods: For this purpose, forty eight male rats (Wistar) with mean 175±25 gr were purchased from Pasteur institute, Tehran, Iran and acclimatized to the laboratory conditions. Then animals randomly divided into four groups each with triplicate. One group selected as control and only received normal saline, another groups fed with L. casei and L. paracasei in combination (5×108 CFU/ml) and The doses of %0.5, %1 and %2.5 of raftilose, respectively. Animals daily gavaged with 1.5 ml for 30 days. Sampling was conducted at the day of 0 and 30 for assay the growth parameters (final weight (WD), weight gain (%WG) and specific growth rate (SGR)) of animals.

Results: Results showed the rat that fed with lactobacillus and raftilose in combination had better growth parameters than control group. WD, %WG and SGR in the dose of %2.5 of raftilose statistically ($p<0.05$) was higher than other groups. there was not seen statistical differences at the level of $P<0.05$ between other groups

Conclusion: The findings of the present research reveal that raftilose 2.5% can have a synergistic effect on growth rat.

Keywords: Synergism, raftilose, lactobacillus, rat

P392: **Toxic effects of lead, nickel and cobalt nitrate on rat liver chromatin components**

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Background and Aim: Heavy metals are environmental pollutants and their application in developing technology and citizens have increased, although we can't ignore the side effects of these metals. Some examples of heavy metals are cadmium, lead, nickel, arsenic, cobalt and etc. Aim: In the present study the effects of lead, nickel and cobalt on chromatin proteins in alveolar macrophages and hepatocytes nuclei was investigated Materials:

Methods: using SDS and agarose gel electrophoresis, UV-Vis spectroscopy and western blotting techniques. Alveolar macrophages were prepared from rat lung by lavage and then incubated with various concentrations of metals

Results: The result showed that viability of the cells were considerably decreased as metals concentrations were increased. The electrophoresis of histone and non histone proteins showed that the histone proteins remained unchanged in the presence of metals although some minor effects were observed. Analysis of DNA extracted from the treated cells and the controls on agarose gel and also using fluorescent dyes reveal that all These metals, especially lead and nickel, at low concentration proceed the cells into apoptosis, as chromatin condensation and DNA fragmentation is occurred in some cells. Whereas at higher concentrations of metals necrosis was occurred. Studies on the effect of metals on nuclei represented that the absorbance at 260, 230 and 210 nm is decreased as metal concentration is increased. SDS gel electrophoresis and western blot also confirmed the results indicating that interaction of metals with the nuclei decrease the extractability of the histone proteins of the chromatin. Moreover the content of non histone proteins (HMG proteins) was also changed in a metal dose dependent manner

Conclusion:: From the results it is finally concluded that lead, nickel and cobalt bind to chromatin proteins, however, the extent of binding is different between the metals.

Keywords: Toxic effects, nickel, nitrate, rat liver

P393: **Prevalence of gasterointestinal parasites in referred cases to Mehrin laboratory in Tehran**

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Background and Aim: Infections by gastrointestinal parasites ,always have been one of the most important of health problems in different society even in development country. Global distributions of parasitic infection due to poverty ,malnutrition , Illiteracy , excessive increase in the population, lack of medical facilities , etc. is responsible for the major of the problems. Role of parasitic disease in economic and social loses in some part of the world is comparable with some of disease as tuberculosis, venereal disease and acute respiratory infection .Generally loses due to these disease affect developing country more than industrial country. The aim of this survey is determine rate and variety of gastrointestinal parasites in Mehrin laboratory as one of the diagnostic center of Tehran.

Methods: This study was carried out during Jun to September 2012. Totally 1329 sample after reception in laboratory and record information as age, sex , location , job , etc. were examined directly.

Results: 15 sample of 1329 samples were infected by gastrointestinal parasites (1.13%) that include 10 (66.66%) Giardia, 3 (20%) Entamoeba hystolitica and 2 (13.33%) were positive for Entamoeba coli. According to chi-square test, difference was considered statistically significant between men and women. Infection in men was more than women ($p<0.05$) and also there weren't any significantly difference according to location (urban or rural), age, job, etc.

Conclusion: According to pathogenic role of parasites for human and their affect on loss of physical, psychological, economic and social and their importance for health study on distribution and infection abundance is necessary in all area and society .It suggested to do more research in this field.

Keywords: Prevalence, Tehran, Parasite, Giardia.

P394: **Molecular detection of Leishmania major in Phlebotomus papatasi the main vector of Zoonotic Cutaneous Leishmaniasis in Khuzestan province**

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Background and Aim: Zoonotic Cutaneous Leishmaniasis (ZCL), caused by a parasite from Trypanosomidae family of the genus Leishmania, has been an ancient endemic disease in Iran. Respectively, Phlebotomus papatasi is the recognized vector of L. major in Iran. In khuzestan, ZCL, due to L. major, is an emerging public health problem, with several cases reported from different parts of the province. The main objective of this study was to investigate molecular identification of the etiological agent of ZCL and identify the vectors of Leishmania major in this region.

Methods: Sandflies were captured from different cities of Khuzestan province using CDC miniature light trap and sticky papers. DNA of the samples was extracted via ISH-Horovize and GenetBio Kit methods. A standard PCR was performed to amplify a fragment of internal transcribed spacers of the ribosomal RNA genes (ITS-rDNA). Positive samples were used in RFLP by BsuRI enzyme for species determination and the positive samples were send for sequencing and the spices of Leishmania infecting those samples were identified firmly using molecular software's

Results: Among 1500 sandflies caught from different regions of Khuzestan province, 198 female P. papatasi were selected as gravid, semi gravid and fresh fed. DNA was extracted from all of them and the ITS-rDNA gene was amplified using standard PCR. About 8 percent of sandflies was infected with Leishmania parasites. After RFLP, sequencing and molecular analyzing using molecular software's, Leishmania major was identified in all sample cases.

Conclusion: Khuzestan province in southwest of Iran is an important focus of ZCL and this is the first molecular study on the disease in the area. According to the results of PCR-RFLP, sequencing and molecular analyzing, L.major infection was confirmed in P. papatasi. This study definitely incriminates P. papatasi as the main vector and L.major as the main causative agent of Zoonotic Cutaneous Leishmaniasis (ZCL) in Khuzestan province.

Keywords: Cutaneous Leishmaniasis, Phlebotomus papatasi , PCR-RFLP, Khuzestan

P395: **Providing a prokaryotic high-level expression system to produce adhesin recombinant protein E of Nontypeable Haemophilus influenzae**

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Background and Aim: Background: protein E (PE) of H.influenzae is an autotransporter that causes adhesion of H.influenzae to epithelial cells and plays an important role in the pathogenesis of the bacterium. It is a 16-17 kD protein and having 160 amino acids. The amino acids 84–108 which constitute the epithelial cell binding region, is completely conserved. Based on the presence of polysaccharide capsule, Haemophyluse influenzae can be classified into 2 major groups: The unencapsulated- (none typable Haemophyluse influenzae) (NTHi) and – encapsulated (typable Haemophyluse influenzae) strains. Both of them have Pe gene with the length of 480 bp in their genome that produce adhesion PE and it is a major pathogenesity factor of H.influenzae and conserved in both NTHi and encapsulated H.influenzae (96.9%–100%). Purpose: The aim of this study is cloning, expression and purification of the protein E as a good candidate vaccine for the further investigation. we provided a prokaryotic high level expression recombinant system (pBAD gIII A- PE- E.coli TOP10) that can produce high level recombinant PE protein.

Methods: Pe gene of NTHi ATCC 49766 strain was amplified by PCR method. The gene was cloned into TA vector (pTZ57R/T) and confirmed by sequencing procedure and then sub-cloned in pBAD gIII A vector and transformed into competent E.coli TOP10. For overexpression, the recombinant bacteria were grown in broth medium containing arabinose.In next step the recombinant protein was purified by using metal affinity chromatography (Ni-nitrilotriacetic acid) (Ni-NTA agarose). The His tag facilitates purification of the recombinant protein by Ni+2-Sepharose resin. Finally The protein was detected and confirmed in SDS-PAGE and western blotting respectively. Further studies to evaluate its immunogenicity is underway.

Results: The cloned gene was confirmed by PCR, restriction digestion and sequencing methods. In comparison between the cloned pe gene and the reference sequences of pe gene in genbank showed 99% similarity. The pe gene was successfully amplified and was confirmed by sequencing. The recombinant protein E was expressed in E.coli TOP10 cells and was purified.

Conclusion: We believe that this is the first report of overexpression of recombinant PE in E.coli TOP10 cells. The protein could be as an vaccine candidate against H. influenza nontypeable and its purification is a crucial step for further in vivo studies.

Keywords: Haemophilus influenza, Protein E, pBAD gIII A

P396: **Visual detection of oligonucleotide sequences using gold nanobiosensor**

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Background and Aim: Although the conventional DNA amplification using PCR in diagnostic laboratories can provide fast results but it's requires skilled personnel and expensive equipment's. To overcome these deficiencies nanoparticles especially gold nanoparticles have been used as ultrasensitive DNA detection that can be used in field applications. Recently, gold nanoparticle was used as label in biomolecular detection particularly in diagnosis of infection disease and bacterial pathogens, instead of conventional molecular fluorophores. These markers had led to improvements in selectivity, sensitivity, multiplexing capacity and made rapid kit test for different pathogens detection.

Methods: In this study, a unique diagnostic probe was designed for the intelligent visually detection of complementary sequences in genome. For this purpose, functional gold nanoparticle probe (AuNP-probe) has been made by attachment 5´-tiolated oligonucleotide to gold nanoparticle surface. After adding complementary sequences to AuNP-probe solution, the hybridization detection was visually capable.

Results: Results showed that upon adding HCl to hybrid AuNP-probe and complementary sequences, the color of the solution remain red, while test samples with non-complementary oligonucleotide led to color change from red to purple, due to acid induced aggregation of AuNP-probe.

Conclusion: The color change of the solution is observed visually by naked demonstrating direct and rapid detection of complementary oligonucleotide. This method requires less than 25 minutes and color change is visible by naked eye. The results of this research can be used to develop novel methods of genomic DNA detection and making rapid and low-cost nanobiosensor for detection of different pathogens.

Keywords: Nanobiosensor, Gold nanoparticle, Hybridization

P397: **Analytical specificity and sensitivity of a Taqman Real Time PCR assay targeting com1 gene of Coxiella burnetii**

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Background and Aim: C.burnetii, the causative agent of Q fever is documented as a severe occupational threat for laboratory stuffs, veterinarians, farmers and zoo and slaughterhouse workers. In addition, the agent is a member of category B of bioterrorism agents. Rapid detection of the agent improves the management of infection in natural or intentional epidemics. In the study, analytical properties of a com 1 gene based Taqman Real Time PCR for detection of Q fever agent was evaluated.

Methods: Specific primers and a FAM and BHQ1 double labeled probe were designed in accord with the com1 gene. Specificity of the assay was tested on genomic DNA of negative control bacteria. Sensitivity of the assay was calculated in serially diluted positive control plasmid containing PCR product of com1 gene.

Results: No amplification signal observed in Taqman Real Time PCR of negative control bacteria accompanied by specific C.burnetii primers and probe. The last tube showing signal of amplification in sensitivity testing, was related to 0.19 fg of the positive control plasmid. Converting the concentration to copy number value, the limit of detection of the assay was calculated 50 copies of the com1 gene.

Conclusion: These results showed high specificity and sensitivity of the Taqman Real Time PCR assay targeting com1 gene of the organism. Further evaluation of the assay using clinical specimens or artificially infected samples is essential to confirm the assay as a valid diagnostic test.

Keywords: Taqman Real Time PCR, Coxiella burnetii, Analytical specificity, Analytical sensitivity.

P398: **Antimicrobial susceptibility of Haemophilus influenzae isolated from helthy children up to 6 years of age in Tehran**

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Background and Aim: Heamophilus influenzae is a fastidious gram-negative coccobacillus which colonizes the human respiratory tract. H. influenzae can be divided to two groups: encapsulated and nonecapsulated H. influenzae strains. The prevalence of antibiotic resistance in H. influenzae strains has been the subject of multinational surveillance studies in several countries. The emergence of resistant H. influenzae strains, particularly ampicillin resistant strains, has limited the routine use of ampicillin and as a result third-generation cephalosporins have become a therapeutic option. Rapid identification of resistant H. influenzae isolates is quite important for the establishment of early and appropriate therapy.According to few reports of antimicrobial susceptibility of H. influenzae strains in our country, the aim of the study was investigation of antibiotic resistance pattern in H. influenzae strains isolated from helthy children up to 6 years of age.

Methods: Samples were collected from nasopharynx of 34 helthy children under the 6 years old in children medical centre in Tehran. Samples were cultured on chocolate agar. H. influenzae were identified according to standard microbiological and biochemical procedures. Molecular confirmation was performed by PCR for omp6 gene which is specific for genus and species. Antibiotic resistance of these strains was determined by Kirby-bauer disc diffusion test for 9 antibiotics including Ampicillin, Cotrimoxazole, Tetracycline, Ciprofloxacin, Ceftriaxone, Chloramphenicol, Erythromycin, Cefotaxime, and levofloxacin.

Results: The rate of resistance of the 34 isolates to antibiotics was as follows: Ampicillin (n=13, 38.2%), cotrimoxazole (n=20, 58.8%), tetracycline (n=31, 91.2%), ciprofloxacin (n=0, 0%), Ceftriaxone (n=4, 11.76%), Chloramphenicol (n=14, 41.2%), Erythromycin (n=16, 47%), Cefotaxime (n=7, 20.6%) and levofloxacin (n=0, 0%). Among the isolates, 8 (23.5%) and 21 (61.7%) of them were resistant to two and three or more antibiotics respectively.

Conclusion: Rapid detection of resistance and early initiation of alternative therapy are important factors in the treatment of meningitis caused by multiple resistance strains. Resistance to Ampicillin and Chloramphenicol has been reported among H. influenza isolates recently. In this research the highest rate of resistance belonged to Tetracycline which is more than other studies. Our results showed that similar to previous investigations, resistance to Ampicillin, Cotrimoxazole Chloramphenicol and Erythromycin should be considered especially in the strains isolated from children. Ciprofloxacin, Levofloxacin and Cefotaxime were more effective drugs against the studied isolates. Monitoring of antibiotic susceptibility patterns of Haemophilus isolates should be continued and prospective studies should be designed to monitor the impact of therapy on the outcome of acute respiratory infection, morbidity and mortality.

Keywords: Haemophilus influenzae, nasopharynx, Antibiotic Resistance

P399: **Rapid Detection of Listeria monocytogenes in Female's Genitourinary Samples by PCR and Fluorescent Probe Method**

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Background and Aim: Listeriosis has a low incidence in humans. However, pregnant women are much more likely than the rest of the population to contract it. Infected pregnant women may have only mild, flulike symptoms. However, infection in a pregnant woman can lead to early delivery, infection of the newborn, and death of the baby. Listeria can proliferate asymptomatically in the vagina and uterus. There are two forms of neonatal infection (granulomatosis infantisepticum): One, an early-onset sepsis, with Listeria acquired in utero, results in premature birth. Listeria can be isolated in the placenta, blood, meconium, nose, ears, and throat. Another, late-onset meningitis is acquired through vaginal transmission, although it also has been reported with caesarean deliveries. L. monocytogenes can often be cultured from the blood, and always cultured from the CSF (Cerebrospinal fluid). Bacteria culture is difficult and time consuming and need at least 5 days. There are no reliable serological or stool tests. New methods of PCR like real time PCR due to using Fluorescent Probe has high sensitivity and specificity. The aim of this research was introducing a fast and easy method for direct detection and screening of microbes that causes Genitourinary infection.

Methods: Standard strain of Listeria monocytogenes was prepared from Pasteur Institute of France (CIP32110) and selected as positive control and PCR was optimized. hly gene as specific gene was selected for detecting this bacteria. GAPDH gene was used as internal control. Clinical samples were selected from Endocervical swabs of women who referred to obstetrician. DNA extraction was done by PCR template purification kit. Vector NTI ver,11 software was used to design a special hybridizing/hydrolyzing probe which is combination of Taq-man and beacon probe. This novel approach was compared with conventional PCR. Fluorescent end point detection method was used for PCR detection by using specific probe and fluorescent detector machine. The FLASH-PCR assay had a detection limit of 5-10 bacterial genomes per reaction when dilutions of genomic DNA from a type strain of L. monocytogenes were tested. For analyzing data, also SPSS software Ver19 was used.

Results: We found that 12.5% of clinical samples were positive by PCR and have L. monocytogenes DNA.

Conclusion: This study showed that this novel approach has high speed, sensitivity and specificity. Because of importance of pregnant women and newborn's health, screening of women in order to control infection is necessary.

Keywords: PCR, hly A gene, fluorescent probe, Listeria monocytogenes

P400: **The Study of prevalence of Borna Disease Virus p40 RNA in a number of Iranian obese patients**

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Background and Aim: WHO has declared obesity to be a global epidemic .obesity seems to be associated with a multitude of factors, but recently Obesity potentially arising from viral infection, 'infectobesity', has largely been overlooked. Increased adiposity has been observed in animal models ,infected with canine distemper virus, Rous-associated virus, human virus adenovirus 36 (Ad36), SMAM and the Borna virus. Ethical reasons preclude experimental infection of human beings with candidate microbes to unequivocally determine their contribution to obesity. In the absence of direct experimental data, robust indirect evidence from several sources and approaches is needed.These indirect evidences have been observed in avian adenovirus SMAM- 1 and adenovirues36. Borna disease virus a nonsegmented, single-stranded RNA virus was identified as a cause of rapid increase of body weight with development of an obesity syndrome without obvious neurological signs in Experimental intracerebral infected Lewis rats. Although BDV is originally an animal virus but some investigation associated it with special neural disorder in human. So all these observation provoked us to survey the possible effect of BDV in human obesity. During the investigation which was operated for the first time in Iran, prevalence of Borna Disease Virus RNA P40 was screened in the peripheral blood mononuclear cells of a number of obese patients (their BMI (kg/m2) were over 30) and normal people as control group via Nested RT- PCR to discuss the possibility of adiposity promoting potential of BDV in human.

Methods: Peripheral blood cells of patients and control group were randomly collected in anti-coagulated tubes from various laboratories of Mazandaran and transfer to the molecular laboratory of Mazandaran University in order to put into analysis by Nested RT- PCR. RNA was extracted from peripheral blood cells using commercial kit(Vivantis – GF-1 Blood Total RNA extraction kit) according to its manufacturer protocol .then reverse transcription was performed. To confirm the quality and quantity of cDNA production, PCR analysis with glyceraldehyde 3 –phosphate dehydrogenase (GAPDH) primers was applied. The PCR products were separated in 1% agarose gel and then stained with ethidium bromide. In this study, in order to diagnose the BDV RNA sequence, we choose BDV P40 RNA coding nucleoprotein as the target, according to its most abundant transcripts in the infected cells and its high adaptation with NNSRNA viruses.

Results: Statistically examination of all these obese patients using Nested RT- PCR technique and then observation of the specific length of P40 bands on 1% agarose gel (the first PCR product is 726 bp and the second one is 447 bp) demonstrated that human BDV RNA is present in the PBMC of some of these obese patients with meaningful and higher prevalence in contract with normal people.

Conclusion: These results confirm that specific viruses such as BDV may relate to human obesity. Consequently new perspective of obesity treatment through anti –virus medication and methods of protection and obesity managements will be replaced the conventional and unsuccessful losing weight methods.

Keywords: Borna Disease Virus ,infectobesity, Nested RT- PCR technique

P401: **The Effect of Pulsed Ultrasound-Induced Physical Stress on the Sensitivity of Gram Positive Bacteria to Some Common Antibiotics**

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Background and Aim: Therapeutic ultrasound as a physical stressor can induce two major types of bioeffects; thermal and non-thermal effects. Cavitation, microstreaming and acoustic streaming are among the main non-thermal effects of ultrasound. In this study, the effect of short term exposure of gram positive and gram negative bacteria on sensitivity of these micro-organisms to some common antibiotics has been investigated.

Methods: Using Kirby-Bauer method for antimicrobial susceptibility testing, the diameter of inhibition zone in ultrasound pre-exposed bacteria was compared to those of non-exposed micro-organisms. For irradiating the culture plates with ultrasound, a 2 minute exposure to pulsed ultrasound (frequency of 1 MHz, intensity of 250 mW/cm2 and duty cycle of 0.25) was used. In the next phase, pre exposed and non-exposed bacteria were transferred to antibiogram disks containg vancomycin, chloramphenicol, nitrofurantoin, ampicillin, ciprofloxacin, and sulfamethoxazole-trimethoprim (SXT).

Results: Findings of this study was unable of showing any difference between the diameter of the inhibition zone in ultrasound exposed and non-exposed gram-negative bacteria. However, in gram-positive bacteria, there was a significant increase in the diameters of inhibition zones of vancomycin, chloramphenicol, nitrofurantoin, and ampicillin.

Conclusion: Altogether, it can be concluded that pre-exposure of bacteria to pulsed ultrasound may increase the sensitivity of these micro-organisms to some common antibiotics. In spite of the fact that the mechanisms of this increased sensitivity are not fully known, this phenomenon may have critical applications in treating antibiotic-resistant infections. On the other hand, more studies are needed to verify if diagnostic ultrasound can induce the same phenomena.

Keywords: Bacteria, Pulsed-Ultrasound, Sensitivity, Antibiotics

P402: **Comparison of Immunohistochemistry and three classical stains for diagnosis of Helicobacter pylori infection in an Iranian population**

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Background and Aim: Helicobacter pylori (H. pylori) is a gram negative microaerophilic bacterium known to be associated with chronic gastritis, peptic ulcer and gastric carcinoma. Hence, accurate diagnosis is essential. A wide array of tests, both invasive and noninvasive, is available for the diagnosis of H. pylori. Pathologic evaluation of mucosal biopsy is widely used to document H. pylori infection. Several studies have shown that Immunohistochemistry (IHC) with specific polyclonal H. pylori antibodies has the highest sensitivity and specificity compared to histochemical stains. But the question of whether IHC should be routinely used regardless of the histologic appearance of a gastric biopsy specimen remains a subject of considerable debate. Therefore this study was conducted to compare Hematoxylin and Eosin (H&E), Giemsa, Toluidine blue and Immunohistochemical staining methods in gastric biopsy specimens with different pathologic grades.

Methods: 54 gastric biopsy specimens were screened for H. pylori infection with H&E, Giemsa, Toluidine blue and Immunohistochemical staining methods. IHC was used as gold standard.

Results: 43 cases were diagnosed on Immunostained slides, while positive cases were found in 18, 24, and 33 cases by using H&E, Giemsa, and Toluidine blue stains. Our laboratory found that Toluidine blue staining method is cheap and easy to use and produces more reliable results than H&E/modified Giemsa methods. In addition, low density and coccoid forms of organisms which were not obvious on histochemical stained slides, were recognized easily by additional analysis with IHC.

Conclusion: In conclusion IHC is highly sensitive and specific for H. pylori, but it should be used in the following circumstances while limiting health care cost: 1. Chronic inactive gastritis. 2. Post treatment biopsies. 3. If the characteristic chronic active gastritis is present but H. pylori organisms cannot be identified by classical stains. 4. Whenever suspicious bacteria that could be H. pylori are seen.

Keywords: Helicobacter pylori, Immunohistochemistry, classical stains.

P403: **Nasal Carrier Rates and Antibiotic Resistance of Staphylococcus aureus**

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Background and Aim: Staphylococcus aureus is an important cause of infection in hospitals. Staff of hospitals who carries S. aureus have important role in nosocomial infections. Nose is the main ecological niche for S. aureus carriers. In this study the frequency of nasal carriage and antibiotic susceptibility patterns of S. aureus were determined among a group of nursery students in Islamic Azad University of Eghlid during April and May, 2013.

Methods: In a cross-sectional study 50 nasal swabs were collected from anterior nares of students (14 males and 34 females) in the range of 19-35 year. The specimens were cultured in blood agar and the grown colonies were tested by gram staining and assessed by catalase and oxidase tests. The positive results were more confirmed by bacterial culture in DNAase and manitol salt agar media to confirm the S. aureus. Antibiotic susceptibility testing was performed by disc diffusion agar method.

Results: S. aureus was isolated from 14 of 50 students (28%) which 4 individuals were male and 10 females from the screened population. No statically significant was shown among the distribution of age and gender in carrier population. The resistance to penicillin and ampicillin were the highest rate (100%) and no resistance to ciprofloxacin and chloramphenicol were noted. For other tested antibiotics, 21% of isolates was resistance for vancomycin. The isolates showed 14% and 58% intermediate sensitivity for gentamicin and nalidixic acid, respectively.

Conclusion: Identification and treatment of S. aureus carriers are important strategies for reduction of nosocomial infections.

Keywords: Staphylococcus aureus, carrier, Antibiotic, resistance.

P404: **detection of Beijing genotypes of Mycobacterium tuberculosis by Melting curve analyze**

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Background and Aim: Tuberculosis is one of the most important infectiousdiseases in the world today.Rapid diagnosis of drug resistant Mycobacterium tuberculosis(MTB) is critical to starting of an appropriate treatment and preventing of more spread drug resistant MTB strains. Due to association of Beijing genotypewith drug resistance in MTB, we developed a rapid and non-culture method for detection Beijing and non-Beijing MTB in clinical samples.

Methods: We modified Taqman Real time PCR for detection Beijing and non-Beijing genotypes of Mycobacterium tuberculosis to a free probe method in presence of a single dye together with amelting curve analysis. We then performed a blinded screening with both methods on 165 septum samples from treated tuberculosis patients

Results: We were obtained the same results by both methods. Of the 165 patients, 30 samples were Beijing genotype and 135 were non-Beijing genotype.In free probe method, we were clearly identified a melting peak at 81°C corresponds to non-Beijing and a melting peak at 88°C corresponds to Beijing genotype

Conclusion: DNA melting curve analysis is a simple and efficient method for the specific detection of amplified products and greatly reduces the cost molecular detection

Keywords: Beijing genotype,Mycobacterium tuberculosis,melting curve analysis

P405: **Rapid detection of katg ser315thr substitution clinical isolates of mycobacterium tuberculosis by using PCR-RFLP**

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Background and Aim: INH, a first-line antituberculosis drug, is very efficient at killing bacilli in the active metabolic state;however, the increase in INH-resistant strains has reduced the efficacy of this drug as a disease control agent in certain populations. Mutations in the katG locus of catalase peroxidase in Mycobacterium tuberculosis (MTB) account for major isoniazid (INH) resistance. Approximately 70–90% of the mutations in the katG gene are located in codon 315, with the most frequent substitution being serine to threonine (AGC: ACC). In this study, a PCR-RFLP method for rapid detection of Mycobacterium tuberculosis clinical isolates resistant to isoniazid was designed and the compared by sequencing results.

Methods: A collection of 80 MTB isolates was used to evaluate the sensitivity and specificity of a PCR-RFLP method for the detection of INH resistance-associated mutations. For preliminary detection of MTB, the PCR assay was performed using the primers based on the katG gene. Each PCR contained 10µl DNA extract. Primers katG F(5'AGCTCGTATGGCACCGGAAC-3') , forward primer and R(5'TTGACCTCCCACCCGACTTG- 3') , reverse primer were used to amplify a 620-bp fragment of katG. A 10 µl aliquot of the PCR product was electrophoresed for 1 h through 2%agarose gel in 1×TAE. For samples positive for the 620-bp fragment of katG, a 5 µl aliquot of the PCR product was digested using 2 U HpaII restriction endonuclease in a 10 µl reaction at 37 8C for 4 h, followed by electrophoresis in a 6% polyacrylamide gel. In this study, the INH-resistance mutation in katG codon 315 (Ser,AGC: Thr, ACC) were identified by RFLP using HpaII digestion (restriction site C: CGG). 20 randomly selected isolates sent for purification and sequencing by an Applied Biosystem apparatus to Souece Bioscience Co.

Results: The katG PCR is highly specific for MTB, in that none of the specimens with MOTT or those that were culture-positive for other respiratory bacterial pathogens showed a positive result. From 80 isolates, 42 strains were phenotypically resistant and 38 ones were susceptible. The katG PCR for a 620-bp amplicon was successful for all purified M. tuberculosis isolates, including standards, susceptible, MDR and XDR isolates (specificity 100%). As a result of molecular method, all of susceptible isolates were non mutant by PCR-RFLP method. In the other hand, 88% of all resistant strains have mutation in katG315. Automated DNA sequencing of the katG amplicon from randomly selected INH resistant isolates verified 100% sequence accuracy of the point mutations detected by PCR-RFLP. DNA sequencing also revealed no mutation other than Ser/Thr315 within the 620-bp katG amplicons for the 20 isolates.

Conclusion: The results of this study indicate that the PCR-RFLP method can be used in routine work as a simple and rapid method for detection of resistance to Isoniazid.

Keywords: Mycobacterium tuberculosis, drug resistance, Sequencing, isoniazid, PCR-RFLP

P406: **Simultaneous detection of tuberculosis and Isoniazid resistance in clinical isolates of Mycobacterium tuberculosis by a molecular method**

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Background and Aim: Early detection and suitable drug admission are the first attempts to control of TB and MDR-TB. In this study, PCR-RFLP method was used for rapid detection of Mycobacterium tuberculosis and resistance to isoniazid.

Methods: Inthepresentstudy,87clinical isolates of MTB consist of 46strainsofdrug-resistantphenotypeand 44susceptible strains were used in a PCR-RFLPmethodby specific primer and HpaII .The RFLP-PCR assay was used for two purposes; In first, PCR of katG gene for diagnosis of bacteria and in second step, PCR product by endonuclease enzyme HpaII in a RFLP reaction produced a pattern in electrophoresis for mutation detection inkatG315. sequencingmethod was used for confirmation of RFLP results.

Results: The study showed that all studied strains produced band 620bpthat confirmed MTB. In addition, from the 46 strains resistant to isoniazid, the RFLP-PCR method showed that 44 strains had mutations in the katG gene Ser315Thr. The 41 susceptiblestrains had not any mutation at the codon. Results of sequencing,were confirmed molecular method results. RFLP-PCR test sensitivity 95.6% (95% CI: 0.85-0 .98) and specificity of 100% (95% CI: 0.91-1 .0) was calculated.

Conclusion: RFLP-PCR test is a good tools for spontaneous detection and diagnosis of katG315 mutation in clinical isolates of MTB.

Keywords: Mycobacterium tuberculosis, diagnosis, drug resistance, katG, PCR-RFLP

P407: **The prevalence of antibiotic resistance in Staphylococcus aureus clinical isolates and the emergence of vancomycin resistance.**

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Background and Aim: To determine the pattern of antibiotic resistance in the clinical isolates of Staphylococcus (S.) aureus, Methicillin Resistant S. aureus (MRSA) and define the possible emergence of Vancomycin resistant S. aureus (VRSA) in Tabriz-Iran.

Methods: Staphylococcal isolates from different clinical specimens, pus, urine, blood, high vaginal swab and other secretions received at Islamic Azad University laboratories were collected. The specimens were inoculated on blood agar, MacConkey agar and Chrom agar. Antibiotic susceptibility to conventional antibiotics was done by disc diffusion, and E-test. Methicillin resistance was tested by using Oxacillin and Methicillin disks and confirmed by gold standard PCR for presence of mecA gene. All MRSA strains were subjected in addition to Vancomycin screen agar test.

Results:: Out of the 350 S. aureus isolates 135 (38.6%) were found to be MRSA. In those isolates, high resistance was found to Cefixime (100%) Doxicycline (100%) Oxacillin (96.5%) Gentamicin, (96.3%), Timethoprim/Sulfametoxazole (95.6%) Chloramphenicol (93%) Tobramicin (81.03%), Ofloxacin (72.4%) and Ciprofloxacin (63.7%). Low resistance was found to Ceftazidine (36%), Amoxicillin/Clavulanate (32.7%), Fosfomycin (31%), Cefroxime (24%), Amikacin (17.2%) and Meropenem (13%). One isolate was found to be Vancomycin resistant (MIC 32 ?g/ml). Four isolates had intermediate resistance, with two strains having MIC of 16?g/ml and two having MIC of 8?g/ml. These strains were also resistant to all the other tested antibiotics except Linezolid to which all isolates were susceptible.

Conclusion: Antibiotic resistance to all the conventionally used antibiotics was high in the tested isolates. All the strains were susceptible to Linezolid which is an expensive alternative with adverse side effects. Judicious use of antibiotics focused on the compliance and formation of antibiotic policy guide lines is highly recommended.

Keywords: Staphylococcus aureus, Methicillin Resistant S. aureus (MRSA), Vancomycin resistant S. aureus (VRSA)

P408: **Comparison of inhibitory effect of ZNO and Agno3 nanoparticle on s.aureus and E.coli**

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Background and Aim:: Hospital acquired infections are a major problem worldwide and controlling the spread of bacteria within a hospital is a constant challenge.according to recent studies, antimicrobial of ZnO and AgNO3 nanoparticle was approved. The results showed that Escherichia coli and Staphylococcus aureus was extremely sensitive to treatment with nanoparticles. In this research antibacterial effect of ZnO and Agno3 nanoparticle on E. coli and S. aureus was investigated.

Methods: Six nylon-clothe samples with 20 by 20 cm2 dimension were prepared and were washed for preparing corona discharge. The corona discharge was done in six different situations. After preparation of ZnO and AgNo3 Nano-particle sample and medium, S.aureus and E. coli were cultured on trypticase soy agar medium and were incubated at 37C for 24h. Finally anti-bacterial power of nano-particles was calculated.

Results: the value of antibacterial activity were appropriated with corona power and deposition that is shown the absorption of ZnO and AgNo3 nanoparticle on samples. Effect of ZnO and AgNo3 nanoparticle on death kinetic of bacteria showed the survival ratio of bacteria decreased with increasing ZnO and Agno3 concentration.

Conclusion: Base on the results, E. coli population at the treatment time decrease faster than S.aureus . Also, the results showed that AgNO3 nanoparticle could be a highly effective disinfect Compared with ZnO nanoparticle for controlling of S.aureus and E.coli .

Keywords: ZnO nanoparticle, AgNo3 nanoparticle, s.aureus, E.coli

P409: **Product ion of anti-helicobacter pylori urease specific immunoglobulin in egg yolk**

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Background and Aim: Helicobacter pylori is a gram negative, spiral and microaerophilic bacteria that infects human gastric mucosa layer. H.pylori, the causative agent of gastritis and gastric ulcer, plays a crucial in development of gastric carcinomas. The importance of hens eggs as a source of specific antibodies (IgY) is well recognized.in this study the UreC of Helicobacter pylori that is recognized by IgY-Hp was identified and used as an immunogen to produce IgY-Hp antibody.

Methods: In this study, hens(25 week, n= 15) were immunized intramuscularly with UreC helicibacter pylori (200 µg/ml, Protein) using an equal volume of frounds complete adjuvant. three booster injection were given at 2-week intervals following the first injection . blood was collected from the hens and serum was isolated and immunization of hens was confirmed by indirect Enzyme Linked immunosorbent assay. On month after immunization the egg laid were collected daily for 1month and store at 4C. IgY obtained from hens immunized with UreC H.pylori was isolated and purified. Purification of IgY was carried out by applying 12% (w/v) poly ethylene glycol (PEG6000).

Results: Indirect ELISA showed high antibody titre in the serum. The IgY-Hp titre against UreC of helicobacter pylori was evaluated using ELISA analysis. SDS-PAGe analysis showed that IgY was produced and purified. The purity of our purified IgY-Hp was 70% with a yield of 50mg of IgY per ml of egg yolk.

Conclusion: Because high level production of these antibody are easy and cost effective .Therefore specific IgY-Hp produced may be an effective tool against infection by H.pylori

Keywords: Helicobacter pylori, IgY antibody , Egg, ELISA

P410: **Genetic diversity of Helicobacter pylori proteases and their distinct proteolytic activities among different clinical isolates**

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Background and Aim: Helicobacter pylori (H. pylori) infection has been strongly linked to peptic ulcer disease and gastric cancer. The secretome of this bacterium may act as important mediators in host-pathogen interactions. The main part of H. pylori secretome is its proteases. So far, there are no available data concerning the prevalence of these enzymes. The aim of present study is screening the prevalence of seven proposed putative protease like proteins of H. pylori isolates, determining their proteolytic activities and their association with pathological and clinical outcome.

Methods: A total of 68 isolates from patients with different gastroduodenal disorders were studied. Molecular screening of the seven proposed putative protease like encoding proteins was determined with designed primers by polymerase chain reaction. Proteolytic activity of each strain was also determined by enzymatic assay, in which Casein used as substrate for all the experiments. Association of the estimated proteolytic activity and genotype diversity of each strain with the severity of gastoduodenal disorders was finally determined by using SPSS statistical package version 19.0.

Results: The prevalence of the seven protease genes: htrA, protease 1012, protease 1350, collagenase, zinc metalloprotease, lipase like protease and protease 1435 were 94.1%, 88.2%, 95.6%, 89.7%, 89.7%, 83.8% and 13.2%, respectively. The results of determination of proteolytic activities showed that 57.4% of the isolates had low, 25% had moderate and 17.6% had high proteolytic activities. The strains which carry protease 1012 gene showed significant associations with moderate active chronic gastritis (P=0.009).

Conclusion: Our results showed that most of the H. pylori isolates could carry most of the proposed putative protease like protein genes. On the other hand, the estimated proteolytic activity showed significant variation among these isolates. In addition Protease 1012 can be proposed as a new virulence factor. These somewhat surprising results shed new light on H. pylori proteases as important mediators in host-pathogen interactions.

Keywords: Helicobacter pylori, protease like proteins, proteolytic activity, Protease 1012

P411: **Evaluation of tetanous toxoid (TT) effect as carrier in immunological of s.aureus Capsular type 8 – tetanus toxoid (TT) conjugate in mice as a candidate vaccine.**

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Background and Aim: evaluation of tetanous toxoid (TT) effect as carrier in immunological of s.aureus Capsular type 8 – tetanus toxoid (TT) conjugate in mice as a candidate vaccine.

Methods: Cp8 extracted by enzyme digestion, then dialysis .To improve immunogenicity, the purified antigen was conjugated to TT with ADH as a spacer and EDAC as a linker. The reaction mixture was passed through a sepharose CL-2B column. Sterility and toxicity tests were shown that prepared conjugate was non-toxic and non-pyrogenic. Then four groups of female BALB/c mice (each group was included 15 mice) was selected. Vaccination was performed by three doses with two week interval. Then serum samples was collected and antibody responses against Cp8 was measured by ELISA method for total IgG, IgG1, IgG2a, IgG2b, IgG3, IgM and IgA. Two week after first dose of vaccination there was no significance different antibody titers between groups that were immunizided by Cp8 and Cp8-TT. But after second and third doses, Cp8-TT showed significance increasing in all types of antibodies titers in versus Cp8.

Results: Overall results of anti Cp8 inductions for total IgG, IgM, IgA, IgG1, IgG2a, IgG2b, and IgG3 were shown: Cp8-TT> Cp8 >TT. The anti Cp8 IgG antibody was predominantly of IgG1 subtype and then IgG3, IgG2a, IgG2b, respectively.

Conclusion: Cp8 from S.aureus increase anti Cp8 antibodies in conjugate form with tetanus toxoid and can be an appropriate effective candidate vaccine for this bacteria

Keywords: S.aureus , Cp8 , Tetanus toxoid , conjugate, ELISA

P412: **PDT combination with Imipenem (1/2 MIC) treatment for pneumonia involving multidrug-resistant Acinetobacter spp**

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Background and Aim: Respiratory infection disease is the main cause of morbidity and mortality in patients with immunosuppressant. In such patients chronic Acinetobacter sp (MDRs) infection is virtually impossible to eradicate using antibiotic therapy. Photodynamic inactivation (PDI) could be one potential alternative antimicrobial method. As photosensitizes could be delivered to the lungs of immunosuppressant patients via inhalation, the current in vitro study investigated the potential use of PDT in the treatment of Acinetobacter spp pulmonary infection. Photodynamic inactivation (PDI) involves the utilisation of photosensitizers activated by exposure to visible light in order to eradicate microbes (this method has already been applied in photodynamic therapy of tumours). Photodynamic effect of the particular photosensitive substance (PS) is attributed to its ability to penetrate susceptible microorganisms, to absorb the light of certain wavelength, and to generate reactive cytotoxic oxygen products. The target microorganisms for photo inactivation are bacteria, fungi, viruses and protozoa.Photodynamic antimicrobial therapy is proposed as a potentially topical, non-invasive approach suitable for treatment of locally occurring infection. The fact that bacteria are becoming increasingly resistant to antibiotics and antiseptics has lead to an increased interest in the development of new alternative eradication methods, such as PDT. chapter will focus on the use of PDT in the treatment of antibiotic-resistant biofilms,isolated from under respiratory tract infections. , afterwards survey sub MIC antibiotic influence on species have been PDT.

Methods:: Effect of photosensitizer concentration (200µM) MB, TBO and laser irradiation time (5 min) on lethal photosensitization was investigated. After PDT have used sub MIC Imipenem concentration on species have been PDT.

Results: When isolates were grown in biofilm, PDI treatment alone was not bactericidal. When PDI was combined with antibiotic treatment, bactericidal activity was apparent. TBO at 200?M, 27 J/cm2, in combination with Imipenem (1/2 MIC) exhibited 3.8 log10 killing for Acinetobacter spp, However, MB at 200?M, 27 J/cm2 , in combination with Imipenem (1/2 MIC) exhibited 4 log10 killing for Acinetobacter spp

Conclusion:: MB diffused more efficiently across Acinetobacter sp than TBO. However, receiver compartment concentrations of Sub MIC Antibiotics after 24 h were of the same order as those required to achieve high rates of kill (>99%) of Acinetobacter sp (MDR) growing both planktonically and in biofilms after PDT.

Keywords: multidrug-resistant Acinetobacter spp,pneumonia,imipenem ,PDT

P413: **Cloning and expression of Staphylococcus simulans lysostaphin gene in Bacillus subtilis WB600**

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Background and Aim: Lysostaphin is a glycylglycine endopeptidase, secreted by Staphylococcus simulans, capable of specifically hydrolyzing pentaglycine crosslinks present in the peptidoglycan of the Staphylococcus aureus cell wall. Cloning and expression of the gene has been reported in various strains. The aim of this study is cloning and expression of the lysostaphin gene in Bacillus subtilis WB600 using pWB980 expression vector.

Methods: Plasmid pACK1 of S. simulans was extracted using alkaline lysis method. Then, the lysostaphin gene was isolated by PCR and cloned into pTZ57R/T Vector and then transformed into Escherichia coli BL21 by cold CaCl2 method. In the next time, the amplified fragment (~750 bp) on pTZ57R/T Vector was digested using PstI and XbaI enzymes. The vector pWB980 was also digested by the same enzymes and purified. The ligation reaction was done between the restricted fragment and vector by standard protocols. The recombinant plasmid was transformed into B. subtilis WB600 by electroporation method at 950 V. The Lysostaphin protein production was estimated by Bradford and SDS-PAGE methods.

Results: In this study, the lysostaphin gene was isolated from plasmid pACK1 of S. simulans. The gene cloning in pTZ57R/T Vector was verified by PCR. The correct ligation orientation was analysed in 9 kanamycin resistant colonies by enzymatic digestion, among which 7 colonies had correct ligation orientation. The size of lysostaphin protein was detected by SDS-PAGE and a band about 27 kDa was seen on polyacrylamide gel. The protein concentration was also assayed by Bradford method and was about 91µg/ml.

Conclusion: This is the first report about cloning and expression of the lysostaphin gene in Bacillus subtilis WB600 using pWB980 expression vector. The amount of expressed protein was suitable in comparison with the similar studies.

Keywords: Lysostaphin, Staphylococcus simulans, Bacillus subtilis, pWB980, SDS-PAGE

P414: **Evaluation of Cytomegalovirus (CMV) in Abortion compared with embryos vaginal delivery by using PCR**

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2- Masoud Laboratory

Background and Aim: This study aims to assess the prevalence of CMV in abortion, compared with vaginal delivery amniotic flouid measured by PCR.

Methods: Samples studied 120 mothers attending hospitals Amiralmomenin, Javaheri and Boali were in 1389, that 60 of them were mothers with abortion (cases) and 60 mothers who had vaginal delivery, have been. All persons after their consent participated in this project and the data field method (PCR) and a questionnaire, to evaluate the microbiological by PCR CMV were that results in the two groups were compared with vaginal delivery and abortion groups and finally, the results by statistical tests and Chi-Square analysis was SPSS13.

Results: For 120 patients, 13 / 3% (16 cases) had positive PCR results, age group, 25-30 years and in the 30-35 years age group and women age group 28/ 5 + 6 / 68 years in the control group and 29 + 5/87 years, the PCR test results, maternal amniotic in the study group, number 26 / 7% of CMV infection and 73 / 3% of cases and controls had a negative PCR test results were reported. Overall, 120 subjects in this study, the incidence of CMV, 13 / 3% respectively. Group, 61 / 7% and 30% of abortion cases, and 8 / 3% had two miscarriages and a history of abortion in the control group, 85% and 15% had an abortion. Group, 21 / 7% of abortions at less than 20 weeks and 78 / 3% took more than 21 weeks (as preterm delivery or intrauterine death) has been and time history of abortion in the control group and 5% in less than 20 weeks and 8 / 3% had a preterm delivery or intrauterine death.

Conclusion: The Chi-Square test between CMV infection and the number of abortions and miscarriages in pregnant women, there is a significant relationship. Recommended that further studies with larger sample size in several centers and compared to other diagnostic methods in order to achieve better results in this regard should be done.

Keywords: CMV, abortion, PCR, Chi-Square

P415: **Phenotypic - lactamaseβand molecular characterization of CTX-M extended -spectrum produced by Escherichia coli isolates in Shiraz**

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Background and Aim: The resistance of Escherichia coli(E.coli) isolates to third generation-cephalosporins such as cefotaxime has frequently been reported since 1980s.These isolates can produce extended –spectrum beta lactamase (ESBL) of CTX-M type. The CTX-M family is divided into five main clusters: CTX-M1, CTX-M2, CTX-M8, CTX-M9, CTX-M25. The aim of this study was to detect the prevalence of CTX-M gene in E. coli isolates in Shiraz Iran.

Methods: A total of 376 of E.coli isolated from 4 teaching hospitals affiliated with Shiraz University of Medical Sciences Shiraz, Iran from January to June 2012. Phenotypic screening and confirmation tests for ESBL were carried out using disk diffusion (Kirby Bauer) method. All of the ESBL producing isolates were tested by PCR using specific primers.

Results: The ESBL phenotype was detected in 202 (53.7%) isolates. Of ESBL producing E.coli, 185 (91.5%) possessed CTX-M β-lactamase genes.

Conclusion: The result of this study showed a high prevalence of CTX-M producing E.coli in Shiraz Iran. This poses a public hazard and grave concern, and it is necessary to take swift and appropriate measures to prevent it.

Keywords: E.coli, ESBL, Shiraz, CTX-M

P416: **Evaluation of ClassII Integron in Pseudomonas aeruginosa Isolated from Clinical Specimens in Yazd, Iran**

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Background and Aim: Pseudomonas aeruginosa is one of the most important causative agents among hospital acquired infections, especially in ICU and burn units.The organism is known to be inherently resistance to a wide variety of antimicrobials. Integrons have been recognized as important contributor to the acquisition and dissemination of antibiotic resistance gene in Gram-negative bacteria. The main objective of this study was to determine the antibiotic resistance pattern and frequency of class II integrons among Pseudomonas aeruginosa strains isolated from patients in Shahid Sodoghi hospital in Yazd.

Methods: This descriptive study was carried out on 144 Pseudomonas aeruginosa referring to Shahid Sodoghi hospital Laboratory from April 2012 to April 2013. Biochemical tests were used for identification of Pseudomonas aeroginosa. The drug susceptibility test, using 8 antimicrobial agents, was performed for all the isolates via agar disk diffusion method. Resistance to three or more classes of antibiotic drug resistance was defined. PCR was carried out for the detection of class II integrons.

Results: Out of144 Pseudomonas aeruginosa strains, 54.9% of them isolated from male. Resistance rates to various antibiotics were as follows: gentamicin (63.2%), imipenem (62.5%), amikacin (58.3%), ceftazidime (56.9%), ticarcillin (55.6%), tobramycin (55.6%), piperacillin (54.9%), ciprofloxacin (48.6%) . 108 (75.3%) isolates were detected as multi-drug resistant. PCR results showed that 22 (15.3%) of P. aeruginosa isolates carried class II integron. A significant correlation was obtained between the presence of integrons and resistance against ceftazidime, imipenem , amikacin and tobramycin ($P < 0.001$).

Conclusion: Optimization of using antimicrobial agents and control of infection is recommended to prevent the increasing population of drug resistant organisms. The antibiotic resistance rates in class II integron-positive strains of Pseudomonas aeruginosa were noticeably higher than those in class II integron-negative strains.

Keywords: drug resistance, Pseudomonas aeruginosa, Class II Integron

P417: **Prevalence of Class I Integron among Pseudomonas aeruginosa in Yazd,Iran**

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Background and Aim: Antimicrobial resistance in Pseudomonas aeruginosa, as one of the most important pathogens commonly implicated in nosocomial infections, has been increased in recent years, moreover the presence of integrons and the associated resistance gene cassettes is well established. The main objective of this study was to determine the antibiotic resistance pattern and frequency of class 1 integrons among Pseudomonas aeruginosa strains isolated from patients in Shahid Sodoghi hospital in Yazd.

Methods: This descriptive study was carried out on 144 Pseudomonas aeruginosa referring to Shahid Sodoghi hospital Laboratory from April 2012 to April 2013. Biochemical tests were used for identification of Pseudomonas aeruginosa. Antibacterial susceptibility test for antibiotics amikacin, cephtazidin, ciprofloxacin, tobramycin, imipenem, ticarcillin, piperacillin, gentamicin was performed using disk diffusion (Kirby- Buer) methods. PCR was carried out for the detection of integrons.Demographic information of patients was registered.Data was statistically analyzed by SPSS V.18.

Results: Out of144 P.aeroginosa strains, 54.9% of them isolated from male and mean age of patients was34.9+22.7(+SD). Burn unit with 68 cases (47.2%) and neurological department 7 cases (4.9%) had the highest and lowest rates of Pseudomonas aeruginosa isolates.Resistance rates to various antibiotics were as follows: gentamicin (63.2%), imipenem (62.5%), amikacin (58.3%), ceftazidime (56.9%), ticarcillin (55.6%), tobramycin (55.6%), piperacillin (54.9%), ciprofloxacin (48.6%) and 108 (75.3%) isolates were detected as multi-drug resistant. PCR results showed that 119 (82.6%) of P. aeruginosa isolates carried class 1 integron. A significant correlation was obtained between the presence of integrons and resistance against gentamicin , amikacin , imipenem and ciprofloxacin ($P < 0.001$).

Conclusion: Our study indicate that Class 1 integrons are widespread in P. aeruginosa isolated from clinical samples. The antibiotic resistance rates in class 1 integron-positive strains of P. aeruginosa were noticeably higher than those in class 1 integron-negative strains.

Keywords: Antimicrobial resistance, Pseudomonas aeruginosa, class 1 integron.

P418: **The prevalence of aminoglycoside-modifying enzyme genes aadB in Pseudomonas aeruginosa Isolated from clinical specimens**

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Background and Aim: Pseudomonas aeruginosa is one of the most important causative agents among hospital acquired infections, especially in ICU and burn units.The organism is known to be inherently resistance to a wide variety of antimicrobials. Enzymatic inactivation of aminoglycosides by aminoglycoside-modifying enzymes is the main mechanism of resistance to these antibiotics in Pseudomonas aeruginosa. The aim Of this descriptive study was detecting the aadB gene among Pseudomonas aeruginosa Isolated from Clinical Specimens in Yazd, Iran

Methods: 144 clinical isolates of Pseudomonas aeruginosa were collected and their antibacterial susceptibility patterns were determined by disk diffusion method for gentamicin, amikacin, tobramycin and kanamycin Disk diffusion method considering CLSI principles. Chromosomal DNA of the isolates was also extracted using salting out and PCR method was used to detect the aadB gene. Demographic information of patients was registered.Data was statistically analyzed by SPSS V.18.

Results: Out of144 P.aeroginosa strains, 54.9% of them isolated from male and mean age of patients was34.9+22.7(+SD). Burn unit with 68 cases (47.2%) and neurological department 7 cases (4.9%) had the highest and lowest rates of P. aeruginosa isolates.The resistance rates, as determined by the disk diffusion method, were as follows: 82% for kanamycin, 63.2 % for gentamicin , 63.2% for tobramycin, and 58.3% for amikacin . The PCR results showed that 114 (79.2%) isolates harbored the aadB. A significant correlation was obtained between the presence of aadB gene and resistance against gentamicin and amikacin ($P < 0.001$).

Conclusion: Considering high prevalence of multi-drug resistant Pseudomonas aeroginosa, it is essential to reduce these pathogens in hospitals through controlling aadB genes transfer.

Keywords: Pseudomonas aeruginosa; Antimicrobial resistance; aadB.

P419: **The prevalence of aminoglycoside-modifying enzyme genes aac (6/)-II in Pseudomonas aeruginosa Isolated from clinical specimens**

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Background and Aim: Pseudomonas aeruginosa (P. aeruginosa) is one of the primary opportunistic pathogens responsible for nosocomial infections. Aminoglycosides are an important component of antipseudomonal chemotherapy. The inactivation of drugs by modifying enzymes is the most common mechanism of aminoglycoside resistance. The aim of this study was determination of the prevalence of aminoglycoside-modifying enzyme genes aac (6/)-II in Pseudomonas aeruginosa isolated from clinical specimens in Yazd.

Methods: This descriptive study was carried out on 144 P. aeruginosa referring to Shahid Sodoghi hospital Laboratory from April 2012 to April 2013. Antimicrobial susceptibility tests (using the disk diffusion method) were performed for all 144 isolates. In addition, all isolates were screened for the presence of aac(6`)-II gene

Results: Out of144 P.aeroginosa strains, 54.9% of them isolated from male and mean age of patients was34.9+22.7(+SD). Burn unit with 68 cases (47.2%) and neurological unit 7 cases (4.9%) had the highest and lowest rates of P. aeruginosa isolates.The resistance rates, were as follows: 82% for kanamycin, 63.2 % for gentamicin , 63.2% for tobramycin, and 58.3% for amikacin . The PCR results showed that 93 (64.6%) isolates harbored the aac (6/)-II .

Conclusion: Markedly high resistance to kanamycin was noted in the present study.Aminoglycoside resistance in P. aeruginosa remains a significant problem in Iran. Therefore, there is considerable local surveillance of aminoglycoside resistance.

Keywords: Pseudomonas aeruginosa; Antimicrobial resistance; aac (6/)-II, Aminoglycoside.

P420: **Recovery of bovine rotavirus infectious particles by gene transfection method in MA104**

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Background and Aim: Rotaviruses, members of the family Reoviridae, are the principal agents of infectious diarrhea among wide range of animal species including mammalian. They cause many deaths per year. Rotavirus consists of three concentric layers of protein and 11 segments of double stranded RNA (dsRNA). Collectively, VP1, VP2, and VP3 and the dsRNA genome construct the core of the virion. VP1 is RNA dependent RNA polymerase and presence of VP2 is necessary for VP1 activity. Previously reported that VP1, VP2 and VP3 are necessary to replicate RNA genome of rotaviruses and produce infectious particles. Aim of this study is to illustrate only VP1 and VP2 proteins are enough for the replication.

Methods: In this study, VP1 and VP2 genes were cloned in a mammalian expression vector, pcDNA3.1(+). Then, cultured MA104 cell line transfected simultaneously by the segmented double stranded genome of rotaviruses and two resulted plasmids. Then, after 60 hours, transfected MA104 cells were fixed and observed under the transmission electron microscope (TEM) by positive staining. Finally, RT-PCR was performed by using standard primers of VP6 for confirmation of virus presence. In three sequentially passages, infection of MA104 cells was done and again the cells were fixed and observed under TEM by positive staining.

Results: Complete rotavirus particles were observed under TEM microscope in positive staining which shows the rotavirus genome could replicate in presence of VP1 and VP2 proteins and VP3 protein is not necessary for the replication. Also RT-PCR of particles proved that these particles are rotaviruses. TEM after third passage showed resulted particles are infectious particles of rotaviruses.

Conclusion: This study illustrates that transfection of rotavirus genome with VP1 and VP2 genes can trigger the replication process of rotaviruses. Furthermore, for isolation of rotavirus, usually filtered stool samples used to infect the host cells. But this new method provides the possibility of mass isolation of rotaviruses in an easier and less cost way. In addition, we can use this method for production and isolation of other viruses with similar structures and functions, and even new recombinant viruses.

Keywords: Rotavirus, Genome, VP1, VP2, Replication

P421: **Prevalence and multi-drug resistance of enteropathogenic Escherichia coli among young children with and without diarrhea in Zanjan**

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Background and Aim: Enteropathogenic Escherichia coli (EPEC) causes sporadic and endemic diarrhea in infants in developing countries. This pathotype is one of the most important causes of epidemics of diarrhea in infants in industrialized countries. The aim of this study was to investigate the frequency of EPEC and their antimicrobial resistance profile in children younger than five years with and without diarrhea.

Methods: In this cross sectional study, 400 stool specimens were collected from children with diarrhea and 150 samples from healthy children younger than 5 years of age with diarrhea hospitalized in Zanjan during 2012-2013. After culture and verifying of isolates by biochemical tests, Antimicrobial susceptibility was performed using the disk diffusion method (Bauer-Kirby) as recommended by CLSI to 13 antibiotics. In order to detect EPEC in diarrheal and control samples, a primer pair's specific for eaeA gene was used in PCR.

Results: A total of 400 children with diarrhea and 150 control children without diarrhea were studied. 140 isolates of E. coli from diarrheal patients and 60 isolates from control group were collected. The frequency of EPEC isolated in diarrheal and control groups were 17.14% (24isolates) and 3.3% (2 isolates), respectively. The most prevalent resistance profile was erythromycin (100%), followed by azteronam 80.7% (113 isolates) and amoxicillin 74.4% (104 isolates). Imipenem was found as an effectiveness antibiotic with susceptible rate of 72.9%. Isolates were resistance to other antibiotics in following pattern: Amikacin (21.4%), Cefoxitine (32.1%), Cefotaxime (50.7%), Ceftazidime (62.8%), Co-amoxiclav (71.4%), Co-trimoxazole (21.5%), Ciprofloxacin (37.1%), Gentamicin (29.3%), Tetracycline (69.3%). Also, 86.4% of isolates were resistant to three or more agents and considered Multidrug resistance (MDR).

Conclusion: The results show that the EPEC strains are the most common E. coli pathotype in the Zanjan. In addition, the frequency of antimicrobial resistance to various antibiotics was high in diarrheagenic E. coli strains. As a result more emphasis on the identification of these organisms is recommended.

Keywords: Antibiotic resistance, Children, Diarrhea, Enteropathogenic Escherichia coli

P422: **The First Investigation of the Gold Nanoparticles Synthesis by Rhodococcus Isolates from Iran.**

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Background and Aim: So far difference methods were developed for synthesis of gold nanoparticles that have wide application in determination and treatment of cancers. Considering the complexity of these methods and the ability to contaminate the biologic environment, applying of microorganisms in nanoparticle production is an interpretable green technology in addition to reducing of the costs Objectives: 1. Studing of presence of Rhodococcos in copper mine soil 2. Studing of the ability for gold nanoparticle synthesis by isolated from copper mine soil

Methods: 30 samples were gathered from copper mine soil field. Following the isolation of Rhodococcos from soil samples using segregator culture medium , they were mixed in liquid medium of MPGY in 27 and PH=7 for 48 hours. The biomass resulted in centrifugation, then 3 gr of the biomass was mixed in 50 ml of HAucl4 (10-3) solution for 24 h in 27 and PH=7 at 200rpm. The date was analysed using electronic microscope of SEM.

Results: After 24 h the colour of biomass was turned from white to purple-crimsone. Which according to the control samples indicates ion reduction of Au3+ to the metal gold Au 0. the Semi graph indicates the preserve of gold nanoparticles in bacteria

Conclusion: The studied Rhodococcos are one of gold nanoparticle synthesis candidates in biotechnology field.

Keywords: Rhodococcos. gold nanoparticle. gold nanoparticle

P423: **Affect produced microbial metabolites nano drug by native bacteria Streptomyces ABRIINW in phagocytosis activity and monocyte macrophage differentiation in human acute promyelocytic leukemia**

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Background and Aim: Despite useful chemotherapeutic agent's discovery in recent five decades, because of the drug resistance in cancerous cells, the need for more effective anti- cancer drugs remains. In the course of screening for anti- cancer agents, in this study we isolated ethyl acetate soluble metabolites from Iranian soil bacteria Streptomyces ABRIINW and investigated its effects on growth and differentiation of human acute promyelocytic leukemia NB4 cell line.

Methods: After isolation and culture of Streptomyces bacteria, ethyl acetate soluble metabolites extracted and NB4 cells treated by various concentrations of these metabolites (10- 100 ng/ml) for 12- 48 h intervals. Trypan blue exclusion test, latex particle phagocytosis assay and Wright- Giemsa staining used for study of anti- proliferative activity and cell differentiation, resptiecvely

Results: Ethyl acetate soluble metabolites exhibited growth inhibition in NB4 cells in dose- and time-dependent manner ($P < 0.05$). Latex particle phagocytosis assays and Wright- Giemsa staining revealed differentiation toward monocyte/ macrophage

Conclusion: As stated, due to the growth inhibitory and differentiating effects of ethyl acetate soluble metabolites, it is hope that these metabolites could be a good candidate for pharmaceutical researches and by designation of nanoformulated drug of it we could more effectively improve the differentiation therapy of cancers.

Keywords: Cancer, differentiation, natural products, Streptomyces

P424: **The prevalence ofESBLstrains ofPseudomonas aeruginosaof patientsadmitted tohospitalAlzahra inesfahanthyme plant in 1391 compare itCompared to those of Zatariamultifloraand Peganumharmala**

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Background and Aim: Purpose: Pseudomonas aeruginosa is an important bacterial pathogens causing nosocomial infections are resistant to many antibiotics is essential. The aim of this study was to evaluate the prevalence of multi-drug resistant strains of Pseudomonas aeruginosa sensitivity and wide spread(ESBLs) in antibiotic-producing ESBLs isolated from clinical specimens Impact thyme extract, herbal impact on quality Compare How effective are herbal antibiotic resistance.

Methods: Methods: An empirical study on 76 cases of Pseudomonas aeruginosa isolated from hospital patients were do in Alzahra hospital. Antimicrobial susceptibility testing of eight antimicrobial agent, was performed according to CLSI criteria and ESBLs producing strains were confirmed by Double Disk Diffusion Testing. Resistance to three or more than three classes of antibiotics was defined as resistance to multiple drugs. Minimum inhibitory concentrations of antibiotics, imipenem, meropenem, cefotaxime, ceftazidime and aztreonam agar dilution method performed by extract plants were Percolation

Results: Results: Pseudomonas aeruginosa isolates were the most resistance against piperacillin, imipenem, cefotaxime / ceftriaxone, gentamicin, ceftazidime, aztreonam and ciprofloxacin was observed. 8 strains (21%) ESBLs were positive. 02% of the samples, at least three kinds of antibiotics, resistant strains and 8 of the 51 samples from endotracheal secretions, 2 wounds and 2 strains isolated from 10 samples of five blood samples MDR, respectively. Among the extracts,Zatariamultiflora was more effective thanPeganumharmala.

Conclusion: Conclusion: This study showed that the prevalence of multidrug-resistant Pseudomonas aeruginosa in clinical samples of the hospital environment with high humidity. Also, imipenem resistance in Pseudomonas aeruginosa isolates resistant to multiple drugs is high. This is a serious alarm for the use of infection control measures to prevent further of spread these microbes are coping infants by herbal medicines.

Keywords: ESBL, multidrug-resistantPseudomonasaeruginosa, plant extracts

P425: **Prevalence of toxin genes in serologically approved, eae (Negative)/bfp (Negative) Enteropathogenic Escherichia coli strains**

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Background and Aim: Enteropathogenic Escherichia coli (EPEC), one of the six E.coli diarrheagenic pathotypes that produce characteristic histopathology known as attaching and effacing (A/E) on intestinal cells. The eae gene which is located in the 'locus of enterocyte effacement' (LEE) pathogenicity island and the bfpA gene, located on a plasmid called the EPEC adherence factor (EAF), have both been used for identification of EPEC and for subdivision of this group of bacteria into typical and atypical strains. EPEC do not produce Shiga, Shiga-like, or verotoxins but have been detected two toxin genes in some EPEC strains which have previously been reported as putative virulence factors in other E. coli diarrheagenic pathotypes. The aim of the present study was to examine prevalence of the toxin genes (astA and ehxA) and their potential association with diarrhea in strains of E. coli that are categorized into EPEC serologically, but are eae-/bfp-.

Methods: We examined a total of 70 collected strains of EPEC serologically approved, which were isolated from 34 children with diarrehea and 36 asymptomatic children in three Iranian provinces, Tehran, Ilam and Mazandaran. DNA of strains were extracted by phenol-chloroform method. The presence of toxin genes (astA and ehxA) was examined by PCR using primers and standard conditions. Data were analyzed by SPSS and chi square testes, and P- value of < 0.05 for genes potential association with diarrhea in EPEC strains.

Results: PCR weres positive for the astA gene in 38 (54.28%) of 70 strains. None of the strains showed presence of ehxA. The result of SPSS and chi square analysis, showed that none of the genes were associated with diarrhea.

Conclusion: In previous studies, astA and ehxA were found in some atypical EPEC strains which in some of those, the association of genes with diarrhea were significant. In this study, prevalence of astA was common, whiles non of the strains showed ehxA gene. The source of ehxA gene is enterohaemorrhagic Escherichia coli specifically EHEC O157: H7. Since EHEC O157: H7 is very rare in Iran, Thus absence of ehxA in EPEC is acceptable. However, further epidemiological studies from other region of Iran are necessary in order to find out of the virulence properties of these strains and establish the exact role of virulence factors in diarrhea.

Keywords: Enteropathogenic Escherichia coli, toxin genes, diarrhea

P426: **Study of inhibitory effects of Satureja khuzestanica against MDR isolates of Pseudomonas aeruginosa**

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Background and Aim:: the prevalence of the inherent drug resistant and acquired relative to antibiotics in the treatment of Pseudomonas aeruginosa infections caused serious problems in treatment it. For in reasons causes the use of alternative medicines instead of synthetic antibiotics is rising. According to many being native medicinal plants in Iran as well as antibacterial effects that they have, the use of these plants are very efficient benefit. In this study the effects of antibacterial Satureja khuzestanica against Pseudomonas aeruginosa has been examined.

Methods: in this study of plant essential oils and solvents that dimethyl solfuksaid (DMSO) has a name, which is used, with a ratio of 1 to 10 and have been combined these together in different volumes has been added to the bacterial suspension. Using the method of Disc-Diffusion, were used to determine the minimum inhibitory concentration (MIC), antibacterial effects of Satureja khuzestanica .

Results:: the diameter of the inhibitory zone disk containing Satureja khuzestanica against Pseudomonas aeruginosa were 25 and 23 millimeters, that confirmed effects inhibitory Satureja khuzestanica. based on the results of a review of anti-microbial Satureja khuzestanica was determined That has MBC about 16 µg/ml against Pseudomonas aeruginosa.

Conclusion: This study revealed that Satureja khuzestanica have antibacterial effects against Pseudomonas aeruginosa. Due to the increasing resistance to antibiotics in Pseudomonas aeruginosa and the economic cost highlight it as treatment supplement. Because Satureja khuzestanica essence has Inhibitory effects against bacteria therefore can use it to inhibit gene expression and therapeutic supplement in patients.

Keywords:: inhibitory effects, Satureja khuzestanica , MDR, Pseudomonas aeruginosa

P427: **The prevalence of lytA gene among Streptococcus pneumoniae isolated from children under 6 years of age in Tehran .**

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Background and Aim: Streptococcus pneumoniae is a major pathogen and colonizes the nasopharynx of human and can also cause otitis media, pneumonia, bacteraemia, and meningitis. The organism produces a range of colonization and virulence factors including the polysaccharide capsule, surface proteins and enzymes .Major virulence factors belonging to the CBPs (choline binding protein) are the autolysins lytA, lytB and lytC.These autolysins hydrolyze murein of the cell wall .Autolysins (lytA) are enzymes that degrade the peptidoglycan backbones of bacteria resulting in cell lysis. The lytA plays a role in virulence through release of highly inflammatory cell wall degradation products and also release of pneumolysin from the cytoplasm.The purpose of this study was to determine the presence of lytA gene among Streptococcus pneumoniae.

Methods: This cross-sectional study from 2010 to 2011 was carried out on 100 strains of S. pneumoniae isolated from nasopharynx of healthy children under 6 years of age in Tehran. Isolates were identified as S.pneumoniae based on colony morphology, susceptibility to optochin, sodium deoxycholate solubility and presence of cpsA gene as a specific gene for diagnosis of S.pneumoniae .All DNA isolation were performed with Kiaspin PCR Template Purification Kit. The lytA and cpsA genes were investigated by PCR assay using specific primers.

Results: All of 100 isolates were alpha-hemolytic on blood agar, susceptibile to optochin, sodium deoxycholate soluble.All of isolates identified by phenotypic detection harbored cpsA gene. Of the total, 86 isolates harbored by lytA gene. Forty one (47.6%) lytA positive strains isolated from female and 45 (52.4%) were from male.

Conclusion: Our results showed the cpsA gene is one of the major index genes for identification of S.pneumoniae.In this study frequency of lytA gene in healthy children of Tehran is higher than other countries and 52 of 86(60.4%) positive cases of lytA gene belong to helthy children under 2 years of age .New method sush as Real-Time PCR using this target gene can improve identification of S.pneumoniae in the samples.

Keywords: S. pneumoniae, lytA gene, frequency

P428: **Assessment of a microplat dilution method for determining the antimicrobial effects of Myrtus communis L. from Iran**

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Background and Aim: The increasing number of bacterial and fungal strains that have developed resistance to classical antibiotics has intensified the search for new antibiotic compounds. Some of the newer discoveries include the numerous antimicrobial peptides produced by animals, plants, and microorganisms. Such substances are thought to have more half life and fewer side effects. On the other hand traditional antibacterial activity methods can be costly, time consuming and the measurements tend to be more qualitative than quantitative. In this paper we have investigated the antimicrobial properties of the essential oil of Myrtus communis L. against microbial pathogens of clinical importance including Escherichia coli, Pseudomonas aeruginosa, B. subtilis, Mehticillin-resistant Staphylococcus aureus (MRSA), Salmonella typhi, ,Staphylococcus aureus, Candida albicanse using microplate assay .

Methods: Methanolic extract of M. communis L. was prepared using maceration method and after concentrating, different concentrations of the extract (50, 25, 12.5, 6.25, 3.125, 1.56, 0.78, 0.39, 0.195, 0.097, 0.049 mg/ml) were used for their antimicrobial effects in microplate. After incubation for 24 h, MIC was measured by MTT as tetrazolium salt (40mg/ml).

Results: MIC results (were evaluated by standard Microplate serial dilution method to) showed that the highest antimicrobial activities against S. aureus, (MIC 0.195 mg/ml), MRSA and P. aeruginosa (MIC 3.125mg/ml) and the lowest antimicrobial activity was observed in cases of C. albicanse (MIC 50mg/ml).

Conclusion: These results indicate that this methanolic extract has appropriate antibacterial properties. Therefore, it can be suggested to combine this medical plant with other agents for clinical applications. Because of rapidly, high accurately and simultaneously analyze of small volumes of multiple samples in microtitre assay, this article suggests using of method for a wide range of antimicrobial effects of medicinal plants.

Keywords: communis L., Pathogenic microbes, Microplate dilution method

P429: **Antimicrobial activities of Dracocephalum polychaetum Bornm. extract against pathogenic microorganisms**

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Background and Aim: Medicinal plants have been screened for their potential uses as alternative remedies for treatment of many infections. Medicinal plants are very important source for discovery of new agents for treating various ailments related to microbial infections and Dracocephalum polychaetum in folk medicine of Kerman used for infection diseases of gastrointestinal tracks. So in this study we evaluated the antimicrobial characteristics of methanolic extract of D. polychaetum against seven pathogenic microbes by microplat dilution assay.

Methods: Methanolic extract of aerial parts of D. polychaetum was prepared using maceration method. The concentrations of 50, 25, 12,5, 6.25, 3.125, 1.56 and 0.78 mg/ml were used for their antimicrobial effects. The concentrations were affected on Escherichia coli, Pseudomonas aeruginosa, B. subtilis , Mehticillin-resistant Staphylococcus aureus (MRSA), Salmonella typhi, Staphylococcus aureus, Candida albicanse by using microplat dilution assay. MIC were measured by MTT as tetrazolium salt (40mg/ml).

Results: D. polychaetum was highly active against S. aureus and MRSA (3.125, 6.25 mg/ml) and the lowest antimicrobial activity was observed in cases of E.coli (MIC 50 mg/ml).

Conclusion: These results indicate that D. polychaetum has appropriate antimicrobial properties. Therefore, it can be suggested to combine this extract with other agents for the preservation of foods against pathogenic and toxigenic microorganisms.

Keywords: Dracocephalum polychaetum Bornm., Pathogenic microbes, Microplat dilution method

P430: **Detection of virulence genes and antimicrobial susceptibility in uropathogenic Escherichia coli isolates of human**

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Background and Aim: Urinary tract infections (UTI) are some of the most common extra-intestinal disease. Uropathogenic Escherichia coli (UPEC) is the most frequent agent causing UTI in human. The severity of UTI is intensified by the existence of a wide range of virulence factors which participate in different aspects of invasion. Improper use of antibiotics causes the drug resistance in E. coli. Researchers have shown that acquisition of resistance might be associated with the loss of virulence factors. The purposes of this study were to determine of the virulence genes and antibiotic resistance profile of Escherichia coli isolates from human extraintestinal diseases in Kerman city.

Methods: Ninety six urine samples were obtained from admitted patients to four different hospitals during October to December, 2009. Isolation and biochemical identification of E. coli specifically was targeted in the isolates. Standard biochemical tests and bacteriological methods were used to confirm the E. coli strains. From each sample one confirmed E. coli isolate was selected and stored in Luria-Bertani broth (In vitrogen, Paisley, Scotland) with 30% sterile glycerol at -70°C. E. coli reference strains 28C (hly+), J96 (sfa/focDE+,papEF+) were used as positive controls and E. coli strain MG1655 was the negative control. All the reference strains were from the bacterial collection of Microbiology Department of Ecole Nationale Vétérinaire Toulouse, France. The antimicrobial susceptibility of E. coli isolates were examined by disk diffusion method according to Clinical Laboratory Standards Institute (CLSI) with Commercial antimicrobial disks (Mast.Co., UK). The antibiotic disks that used in this study were cefazolin (30 µg), ciprofloxacin (5 µg), co-trimoxazole (1.25/23.75 µg), nitrofurantoin (300 µg), gentamicin (10 µg), imipenem (10 µg), cefepime (30 µg) and nalidixic acid (30 µg). E. coli ATCC 25922 was used as a quality control strain.

Results: PCR assays revealed that 95 (99.15%) E. coli isolates exhibited at least one of the examined virulence genes. All of the detected genes were present alone or in combination with others. Out of 96 examined E. coli isolates 41(42.8%) isolates were positive for iucD gene which was the most prevalent genetic marker. Among positive isolates for iucD, 16 (28.58%) isolates exhibited the gene alone and 25 (14.22%) isolates were in combination with sfa, pap and hly genes. The genetic marker sfa was found in 34 (35.5%) isolates, which was the second most prevalent virulence gene. All of the sfa positive isolates had one of the other examined genes. Among positive P fimbriae encoding gene, 18 (18.75%) isolates were in combination with other genes. There were two (2.1%) positive hly genes which were in combination with three others.

Conclusion: Escherichia coli is the most common cause of urinary tract infections. Different virulence factors have been involved in E. coli infections. Based on this study, genetic marker of iucD gene (42.8%) was the most prevalent gene. Antibiogram results showed that the minimum antibiotic resistance rates was against imipenem (0%) and nitrophorantoin (6 %) and the maximum antibiotic resistance was against cephazolin (91%) and cefepim (76 %).

Keywords: virulence genes, Esherichia coli., antibiotic resistance, Kerman

P431: **Phototoxicity of phenthiazinum dyes against Pseudomonas aeruginosa biofilms treated with acetylcystein**

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Background and Aim: Bacterial biofilms cause chronic infections because they show increased tolerance to antibiotics and disinfectant chemicals as well as resisting phagocytosis and other components of the body's defense system. Pseudomonas aeruginosa biofilms are involved in the pathogenesis of ventilator-associated pneumonia, peritoneal dialysis catheter infections, bacterial keratitis, otitis externa and burn wound infections. P. aeruginosa lung infection in cystic fibrosis patients is caused by biofilm-growing mucoid strains. The increase in resistance to antibiotics among disease-causing bacteria necessitates the development of alternative antimicrobial approaches such as photodynamic inactivation (PDI). PDI uses light in combination with a photosensitizer to induce a phototoxic reaction. Acetylcysteine (AC) is a mucolytic agent. AC was found to decrease biofilm formation by a variety of bacteria and reduces the production of extracellular polysaccharide matrix while promoting the disruption of mature biofilms. Since the antimicrobial susceptibility of biofilm–associated bacteria is enhanced in disrupted biofilms, it is conceivable that an antibiofilm/antimicrobial agent combination would be synergistic. So, the aim of this study was to evaluate the in vitro bactericidal effect of methylene blue/toluidine blue O-PDI (MB/TBO-PDI) on P. aeruginosa biofilms treated with AC.

Methods: Seven biofilm-producing multi-drug resistant P. aeruginosa, isolated from wound infections, were used in this study. Effect of photosensitizer (MB/TBO) concentration (200μM) and light irradiation time (10 min) on PDI of P. aeruginosa biofilms treated with AC (10 mg/ml) was investigated.

Results: When exposed to AC only, there was no statistically significant decrease in log10 viable count, in comparison with the untreated control, for each 7 biofilm-grown strains ($p > 0.05$). MB-PDI reduced viable cell counts by > 1 log10 in the pre-formed biofilms of all tested strains, while, MB-PDI applied on biofilms treated with AC showed the highest ability to disrupt pre-formed biofilms (reduction the number of viable cell counts by 3.8 log10 in comparison to controls in all tested organisms). The applied TBO-PDI on pre-formed biofilms treated with AC decreased viable biofilm-associated bacteria (4.3 log10) relative to the control ($p < 0.05$).

Conclusion: By degrading the extracellular polysaccharide matrix of biofilm, it is possible that AC may have made the biofilm-associated bacteria more susceptible to phototoxic effect of MB/TBO. According to our data, to improve the efficiency of PDI using MB/TBO on Pseudomonas aeruginosa growing as biofilms, acetylcystein could be a promising agent.

Keywords: Pseudomonas aeruginosa, biofilms, photodynamic inactivation, acetylcysteine

P432: **Identification and production of antilisterial enterocin from Enterococcus spp**

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Background and Aim: The Enterococci are lactic acid bacteria (LAB) which form an important part of environmental, food and clinical microbiology that produce a group of ribosomally synthesized antimicrobial peptides or bacteriocins, known as enterocins, Enterocins are peptides with antimicrobial activity synthesized by Enterococcus species, such as E.faecalis, E.faecium, E.durans and E.munditi. Many of these enterocins show strong bacteriocide activity against microorganisms such as Listeria monocytogenes, Staphylococcus aureus, Clostridium spp., E.coli, Vibrio cholera and Bacillus cereus, in food. The increased consumption of foods containing additives formulated with chemical preservatives and consumer concerns have created a higher demand for more natural and minimally processed foods. This interest and also the potential applications in health care sectors have attracted the interest of academia and industry resulting in increased numbers of published research on bacteriocin production. As a result, our aim of this study was isolation and screening for antilisterial enterocins production by enterococci isolated from vaginal swabs.

Methods: A total of 198 vaginal swab samples were taken from previous spontaneous abortion in Lorestan, Iran. Identification of the bacteria was carried out on the basis of its morphological, biochemical characteristics. To detection of antilisterial activity of cell free supernatant enterocins disk diffusion method was used. The protocol used, blank strile diskes treated with 10 µl of 0.5 MacFarland standard of Enterococcus species were placed on BHI agar plates seeded with the same concentration of Listeria monoasytogenes. The plates were checked for zones of inhibition surrounding te entrococcus colonies after incubation at 37°C for 24 h. Antibiotic susceptibility assay for vancomycin and teicoplanin was perforemed by disk diffusion method on Mueller hinton agar according to CLSI breakpoint.

Results: A tolal 126 Entrococcus isolated that, 49 (38/8%) among them inhibited the growth of Listeria monoasytogenes (ATCC 13932) in different activity. seven (24%) E.faecium, 2 (4/08 %) E.faecalis harbored strong antilisterial activity (≥13mm) and 12 (24/49%) E.faecium, 28 (57.14%) E.faecalis showed weak antilisterial activity (9-12mm). hemolysis activity showed that Among these isolates, 12 (24/4%) E.faecium and 14 (28/5%) E.faecalis strains displayed a γ-haemolytic activity, indicating absence of haemolysis. Seven (14.3%) and 1 (2%) were resistance to teicoplanin and vancomycin, respectively. However, 7 isolate that showed strong antilisterial activity were completely sensitive to vancomycin and teicoplanin.

Conclusion: According to our results, Among isolates with strong antilisterial activity seven (24%) E.faecium , 2 (4/08 %) E.faecalis harbored strong antilisterial activity (≥13mm) and had well potential for enterocins production. Bacteriocins produced by LAB have the potential to cover a very broad field of application, including both the food industry and the medical sector Nevertheless, the safety and implications for public health of individual enterococcal strains must be carefully evaluated to fully exploit their industrial potential.

Keywords: Enterococci, antilisterial, enterocin

P433: **Evaluation of metallo-β-lactamases genes in Pseudomonas aeruginosa isolated from Tehran hospitals with phenotypic and genotypic method**

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Background and Aim: Pseudomonas aeruginosa is an opportunistic human pathogen and an important agent for hospital acquired infections especially in immunocompromised, cystic fibrosis, neutropenic, burned and AIDS patients. For treatments of infections caused by this organism, antibiotics such as aminoglycosides, wide-spectrum cephalosporines and β-lactamase often used. Production of class B β-lactamase including IMP and VIM types that are generally referred to as Metallo β-lactamase has become a major obsticle in treating Pseudomonas infections.

Methods: A total of 212 P.aeruginosa strains were isolated from various clinical cases in 9 hospitals in Tehtan during 6 months period in 1390. After performing initial bacteriological tests, they were confirmed to be P.aeruginosa by PCR assay. Their antibiotic sensitivity patterns were initially determined by disk diffusion method against Imipenem, Ciprofloxacin, Ceftazidime, Cefotaxime, Gentamycin, Tobramycin, Piperacillin, Ticarcillin, Azithromycin and Tetracycline antibiotics. Minimal Inhibitory Concentration (MIC) levels for the resistant antibiotics were subsequently determined. A total of 100 isolates which were resistant to Imipenem were selected for analysis of metallo β-lactamases genes by PCR. The Double Disk Synergy Test (DDST) phenotypic assay was initially used to confirm production of metallo β-lactamase. Subsequently DNA extractions were performed and PCR assay using blaIMP and blaVIM metallo β-lactamase gene specific primers were conducted. A metallo β-lactamase producting standard strain was used as positive control.

Results: The percent resistance of the 212 isolates in the initial disk diffiusion method was as follows: Imipenem (47/16%), Ciproflixacin (48/81%), Ceftazidime (46/69%), Cefotaxime (62/26%), Gentamycin (51/41%), Tobramycin (50/47%), Piperacillin (53/77%), Ticarcillin (60/37%), Azithromycin (46/69%) and Tetracycline (86/32%). The MIC method indicated that 43/39% of the strains were resistant to imipenem. In the Double Disk method performed on the 100 Imipenem resistant isolates, 70 strains were shown to be positive which is indicative of phenotypic production of metallo ?-lactamase. The PCR assays indicated that 20 of these strains contained the IMP gene; whereas, 70 of them harbored the VIM gene

Conclusion: The high rate of antibiotic resistance among Pseudomonas aeruginosa isolates in Tehran hospitals which was detected in this study is very alarming. It necessitate careful use of antibiotics for treatment of Pseudomonas infections.

Keywords: Pseudomonas aeruginosa, Metallo β-lactamase, Antibiotic resistance

P434: **Evaluation antimicrobial effect of nano dendrimer with ceftazidime on Pseudomonas aeruginosa**

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Background and Aim: Pseudomonas aeruginosa is a common cause of nosocomial infections and major causes of morbidity. Despite the fact that we live in an era of advanced and innovative technologies for elucidating underlying mechanisms of diseases and molecularly designing new drugs, infectious diseases continue to be one of the greatest health challenges worldwide. Nano dendrimers were able to overcome these issues and facilitate antimicrobial delivery to microbial infection sites. In recent years, encapsulation of antimicrobial drugs in nanoparticle systems has emerged as an innovative and promising alternative that enhances therapeutic effectiveness and minimizes undesirable side effects of the drugs. In addition, both hydrophobic and hydrophilic agents can be loaded into dendrimers. Hydrophobic drugs can be loaded inside the cavity in the hydrophobic core, and hydrophilic drugs can be attached to the multivalent surfaces of dendrimers through covalent conjugation or electrostatic interaction.

Methods: In this investigation for loaded nano dendrimer (PPI) were dissolved in drying THF and then ceftazidim drug molecule was added. amount of the trapped drug was measured weight before and after of conjugation. Nanoparticles size Measure by nanozetasizer experimental study, the activity of nano dendrimer against Pseudomonas aeruginosa was determined by agar well diffusion method. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of nano dendrimers were determined by macrodilution method.

Results: the amount of the trapped drug was 33.33 mol% and size of nanodendrimer-ceftazidime was 156 nm. The results showed that of nano dendrimer-ceftazidime in low concentrations can kill Pseudomonas aeruginosa in laboratory conditions. By increasing concentration of nano-drug antibacterial effect was increased. MIC equal to 0.24 µg/ml and MBC equal to 0.48 µg/ml.

Conclusion: the antimicrobial drugs successfully loaded into dendrimer nanoparticles and have shown improved solubility and therapeutic efficacy.nanoparticle-based drug delivery systems will continue to improve treatment to bacterial infections, especially in life-threatening diseases such as Pseudomonas aeruginosa infections.

Keywords: nano dendrimer, ceftazidime, antimicrobial, seudomonas aeruginosa.

P435: **Nano Dendrimer as carrier ceftazidime for antimicrobial drug delivery to Pseudomonas Aeruginosa**

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Background and Aim: Infectious diseases of the most common diseases in the world and has created many problems for human societies. Pseudomonas aeruginosa nosocomial infection and multidrug resistance to the important factors are considered. Today, novel drug delivery systems (NDDs) are regarded as suitable means for modifying pharmaceutical characteristics of a wide variety of drugs. In the field of drug delivery and particulate carrier systems, antimicrobial drugs have been successfully loaded into dendrimer nanoparticles and have shown improved solubility and therapeutic efficacy. The polycationic structure of dendrimer biocides facilitates the initial electrostatic adsorption to negatively charged bacteria. The absorption then increases membrane permeability and allows more dendrimers for entering the bacteria, leading to leakage of potassium ions and eventually complete disintegration of the bacterial membrane The purpose of this study is, conjugation of ceftazidime with dendrimer nanoparticles to kill Pseudomonas aeruginosa and the study Evaluation of Kinetic Release.

Methods: In this investigation for conjugation nano dendrimer (PPI) with ceftazidime, the nano dendrimers were dissolved in drying THF and then ceftazidim drug molecule was dissolved in THF .The attachment of ceftazidime to nano dendrimer (PPI) was stadied by FT-IR spectroscopy. antimicrobial effect of nano dendrimer carrier ceftazidime on Pseudomonas aeruginosa in Mueller Hinton agar well diffusion method was evaluated.

Results: The size of nanocarriers drugs measured by zetasaizer was 156 nm. Reales prolong was 72 hours without significant burst effect. the Results (MIC) and (MBC) showed that, By increasing concentration of nano-drug antibacterial effect was increased. Nano demdrimer without drug has antibacterial effect.

Conclusion: The use of nano dendrimers as drug carriers by encapsulating antimicrobial is a potential method for delivering highly active pharmaceutical compounds to kill or inhibit the growth of bacterias.

Keywords: nano dendrimer, ceftazidime, seudomonas aeruginosa.

P436: **Characterization of the Modified Hodge test-Positive Isolation of Escherchia coli in Tabriz, Iran.**

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Background and Aim: Carbapenems would be the main therapeutic option for treatment of serious infections. They could used for extended spectrum β-Lactamaese producer and highly resistant Enterobacteriaceae. For first time, there was an ominous report from Japan in a Serratia isolates that encoded for a metallo β -Lactamase gene called IMP-1, in 1995. So, the aim of this study was to evaluate antimicrobial susceptibility against carbapenems in E.coli isolates by disk diffusion agar and Hodge test Method.

Methods: In this study, 96 isolates of Escherchia coli were collected from Imam Reza Hospital, Tabriz, Iran. The isolates were identified by common microbiological methods. Antibiotic susceptibility test was performed for Imipenem, Meropenem, Eratapenem, Gentamaycen, Ceprofloxacin, Nalidixic, Cefteriaxon, Ceftazidim and cefepime by disk diffusion mothod according to CSLI. Hodge test was done for determining carbapenamase in isolates.

Results: Antibiotic susceptibility test showed that resistance rates to imipenem, meropenem, ertapenem, gentamaycin, nalidixic acid, cefteriaxon, cefepime, ceftazidime and ciprofloxcin were 3.1%, 4.2%, 4.2% , 39.6% , 78.1% , 66.7% , 50% , 64.6% and 69.8%, respectively. A total of 3 isolates (3.1%) were cabapenamase producer using modified Hodge tests

Conclusion: Modified Hodge test is a simple test, cost benefit and it can apply for detection of carbapenemase in isolates in the routine laboratory in a less period for isolates showing intermediate or sensitive zone diameter by disk diffusion Agar method.

Keywords: Escherichia coli , Carbapenemase, Hodge test, Antibiotic Resistance.

P437: **Study the Effects of High and Low Frequencies Pulsed Square Electromagnetic Fields on the Logarithmic Growth of the E. Coli**

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Background and Aim: The bacteria are prokaryotic microorganisms, and many species and genera of them are widely distributed in nature. Depending on the beneficial and harmful bacteria in nature are these microorganisms, they have so different effects. E. coli is a bacterium known in this field; it has certain physical properties and cellular and biochemical characteristics. Electromagnetic radiation is a type of energy that can move in space with the speed of light. The quantum radiation as a stream of energy called photons, each photon energy is considered to depend on the radiation frequency.

Methods: The effect of high frequency electromagnetic fields (110 Hz with intensity of 700 milli gauss) and low frequency (10 Hz with intensity of 700 milli gauss) on the bacterium Escherichia coli (ATCC 1533) were studied. In this study, the bacterium E.coli was cultured in BHI broth at 37°C for 24 hours then serial dilutions were made to and from the sixth dilution, one sample was treated with electromagnetic field for six hours and one put aside as control sample.

Results: The results showed a significant increase in the logarithm of the number of E.coli (CFU/ml) treated with high frequency electromagnetic field and a significant decrease in the number of E.coli (CFU/ml) exposed to low frequency electromagnetic field. The results of biochemical tests also showed negative effects of electromagnetic fields on the biochemical properties of Escherichia coli as a bacterium.

Conclusion: Field research was conducted at a frequency of 110 kHz and 700 mG intensity increase 2.4 times the number of bacteria.

Keywords: Electromagnetic Field, Bacterial Growth, E. coli.

P438: **Prevalence of Hand And Nasal Carriage of Staphylococcus aureus among hospital staff and Antibiotic resistance Pattern in educational centers of sari. 2012-2013**

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Background and Aim: Methicillin-resistant Staphylococcus aureus is a common pathogen responsible for health-care-associated infections.Healthcare workers (HCWs) are the main reservoir for Staphylococcus aureus transmission to patients. identification and nasal decolonization in Healthcare workers colonized with S. aureus may aid in the development and reinforcement of infection control strategies .The aims of this study were to identify the prevalence of hand And nasal carriage of Staphylococcus aureus among hospital staff , to analyses their antibiogram with special reference to methicillin resistance.

Methods: This cross-sectional study was conducted in 1391. 148 health workers from different parts of Sari city hospitals participated. Sampling was conducted every individual fingers and Sterile swabs were used to collect the samples from the anterior nares. Samples were cultured on mannitol salt agar immediately .The isolation of Staphylococcus aureus and their antimicrobial susceptibility patterns were carried out by standard bacteriological procedures. Suspected colonies of Gram stain, catalase and coagulase tests were identified. Susceptibility testing was performed by disk diffusion method.

Results: S. aureus carrier status was observed in 24 individuals (16.2%) in this study, and (9.5%) of population were resistant to methicillin. The highest percentage of carriers of Staphylococcus aureus was in the operation room- angiography staff and internal pediatric ward . All strains were sensitive to vancomycin and Chloramphenicol and resistant to Penicillin and Amoxicillin.

Conclusion: The prevalence of the S.aureus carriage in Surrey Educational hospitals staff , compared to similar studies conducted in hospitals in Iran is low. This study showed that the prevalence of MRSA in hospitals and even different departments of a hospital is a significant difference. So appearance and spread of antibiotic-resistant strains can be prevented by removal of predisposing conditions and appropriate use of antibiotics

Keywords: MRSA, Nosocomial Infection, Hand, Nasal

P439: **Investigation of cellular changes in cervix lesions induced by herpes simplex virus(HSV) and molecular detection of virus**

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Background and Aim: Cervix cancer is the second most prevalent cancer in the world and the 12th one in iran .Human Papilloma virus (HPV) is currently one of the recognized agents causing cervix cancer. In this study we investigate the relation between herpes and cervix lesions. The relation between cancer ad Papilloma viruses has been approved. However, since all women suffering from this virus do not experience such malignancy, it might be claimed that this virus is necessary but not sufficient in causing this cancer.in this study we examine that if there is a biological realation between HSV-2(Herpes Simplex Virus-HSV) and HPV-16 and HPV-18 during carcinoma cervix evolution or not.

Methods: The method employed to carry out this study was a retrospective one and to do this, samples of cervix lesions in patients in Khatem-ol Anbia Hospital in Tehran and Dey laboratory in Hamedan were investigated. The samples, after being colored, were examined in terms of the type of lesion in each tissue. The examined lesion types included CINs, Squamous cell carcinoma and Adenocarcinoma. After extracting DNA, PCR was implemented to recognize Herpes simplex virus2 and high risk HPVs, namely 16 – 18 - 31 , by means of designed and commercial primers.

Results: According to the examination of 45 case patients and 15 case controls , the cervix lesions in case patients in order of frequency are CINs that is 68%, Squamous cell carcinoma 23%, and adenocarcinoma 9%. The highest age category involved in this study has been 36-45 and the lowest involved has been 75-66. Of the total case patients, one carried Herpes virus2 and three other had HPV16 and 5 samples had HPV18, and of the total case controls one was HPV16+.

Conclusion: According to the results that we achieved from this study , we conclude that there is a small chance that HSV2 is a cofactor to cervical cancer , but the role of high risk HPV in this type of cancer is significant as was proved in previous studies.

Keywords: Cervical cancer,Human Papilloma Virus,Herpes Simplex Virus

P440: **Using molasses in simultaneous production of propionic acid and acetic acid by Propionibacterium freudenreichii**

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Background and Aim: Molasses which is a by-product of sugar refining plant is used widely as a raw material for fermentation. Due to its abundance and low cost, it can be utilized for reducing production costs. Propionic acid is an important chemical for the synthesis of cellulose fibers, herbicides, food preservatives, etc. Microbial production from agricultural or industrial waste can be a useful way of producing propionic acid economically. The aim of this study was to produce propinic and acetic acid from molasses in fed-batch fermentation simultaneously.

Methods: Fermentation was carried out in a 3-liter fermentor by the use of Propionibacterium freudenreichii sp. shermani at 30 °C for 144 h. Molasses was the initial carbon source and lactose the secondary, which was added to fermatation after 36 h by constant rate of 0.03 L/min. In order to measure biomass and organic acids, samples were taken every 24 hours from the fermentor. Biomass and organic acids were measured by freeze-drying and HPLC method.

Results: Propionic acid production began when the secondary carbon source was added and acetic acid was detected in sample which was taken at 72 h. Final concentration of dry biomass, propionic acid, acetic acid and lactic acid were obtained (grams per liter): 2.1±0.04, 5.57±0.04, 9.31±0.08 and 15.70±0.09. Although it was expected to produce vitamin B12 and folate in this study, but none were detected.

Conclusion: Fed-batch model of propinoic acid production is a good way to obtain significant amount of both propionic and acetic acid. It seems that a simulating agent (e.g. adding different carbon source) is required to shorten the lag phase so that Propionibacterium could produce organic acids at the initial time of fermentation.

Keywords: Propionic acid, Acetic acid, fed-batch fermentation, molasses

P441: **Detection of community-acquired TEM producing klebsiella pneumonia in sanandaj,Iran**

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Background and Aim: The purpose of this study detection of community-acquired TEM producing klebsiella pneumonia.

Methods: We evaluated 100 K.pneumonia from the laboratories of sanandaj city. This gene confirmed by PCR method and we have 88 positive ESBL isolate.

Results: The prevalence of Tem producing- K.pneumonia was found 19(%21).

Conclusion: The results of this study suggest that community acquired need more attention for prevalence of ESBL like TEM.

Keywords: klebsiella pneumonia - ESBL - TEM - PCR

P442: **Survey of frequency in extended-spectrum beta-lactamase producing Enterobacteriaceae and determination of the antibiotic resistant pattern by combination Disk in Ahvaz.**

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Background and Aim: Enterobacteriaceae produce the extended-spectrum beta- lactamases which is considered as an important resistant mechanism of beta- lactam antibiotics. The resistance to beta- lactam antibiotics is the main problem in the bacterial infections therapy. The present study aims to explore the frequency of the extended-spectrum beta- lactamase in isolated bacterias and to determine the isolates' resistant pattern to antibiotics.

Methods: Methods: The totals of 240 isolated Enterobacteriaceae were collected from the clinical samples of Golestan and Imam Khomeini hospitals which were identified by standard biochemical tests. Next, the sensitivity of the isolates to the nine antibiotics was determined with Disk diffusion method. Then, isolated generator Enterobacteriaceae were detected with the combination disk method on the basis of CLSI criteria.

Results: Among 240 isolated bacteria which were detected, E.coli (71.3%) , Entrobacter (27.1%) and Klebsiella (1.2%) have formed the most isolates. According to the results of the phenotypic tests, 108(45%) isolates out of 240 Enterobacteriaceae were beta- lactamase generator. Moreover, the results of diffusion disk have revealed that the resistance of Escherichia coli, Entrobacter and Klebsilla isolates to ceftazidim and cefotaxim were 43.3 and 55.8 percent, respectively.

Conclusion: The current study demonstrates that generator Enterobacteriaceae strains are increasing.Therefore, study of the extended-spectrum beta-lactamase enzymes would help in prescribing the suitable medicine and consequently could prevent the spread of resistant bacteria.

Keywords: Enterobacteriaceae , Extended-Spectrum Beta-Lactamases ,Combination Disk

P443: **Common microbial causes of diarrhea in children less than 5 years in Rasht city**

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Background and Aim: Diarrhea is the major cause of mortality ,malnutrition,physical and mental retardation. In Iran, after respiratory infections, diarrhea is considered as the second leading cause of child mortality . So, diarrheal disease is currently one of the major health issues and the treatment.

Methods: In this cross - sectional study, a total of 240 stool samples obtained from children less than 5 years admitted to the pediatric ward with symptoms of the infection gastroenteritis in 17 Shahrivar hospital. Chromatography using rapid diagnostic kits were addressed E. coli, Salmonella, Shigella, H. pylori ,Rotavirus, Adenovirus, Cryptosporidium parvum, Giardia lamblia. Necessary data were gathered through a questionnaire and statistical software SPSS were analyzed.

Results: The results showed that the highest prevalence rate of microbial agents are respectively: Escherichia coli(34/6%), Adenovirus(26.3%), Rotavirus(16.7%), Salmonella(11.7%), Cryptosporidium parvum(4.6%), Shigella(4.2%), Giardia lamblia(1.7%), H. pylori(0/4%). It was found that the highest prevalence was in the age group under one year is statistically significant (P <0/05).So, between age and the incidence of diarrhea was significantly correlated. In this study, urban residents than rural residents had the highest infection rate and disease prevalence was high in winter. The most common symptoms are nausea and vomiting. The study found children who were not breast feeding,had high risk of pollution. Statistically significant relationship was not observed between sex,drinking water with diarrhea.

Conclusion: This study showed Escherichia coli and Salmonella are respectively considered as cause of bacterial diarrhea in children.

Keywords: Microbial agents,Diarrhea, Children under 5 years,Rasht, Diagnosis chromatography

P444: **In-vivo antibacterial activity compound extracted of bacteria Pseudoalteromonas piscicida PG-01**

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Background and Aim: The aim of this study was the evaluation of invivo effect of antibacterial compound extracted from Pseudoalteromonaspiscicida PG-01 in reducing total bacterial counts in the Murine Thigh infection model

Methods: Six-week-old, specific-pathogen-free, female mice weighing 25±1 g were used for the study. Freshly plated Staphylococcus aureus were cultured in broth media. For providing the injection inoculum with bacterial counts 10 6.6 to 10 8.7 CFU/ml, cultured S. aureus inlogarithmic phase was diluted with a1: 10 ratio in MHB medium. Thigh infections of each isolate were established by injection of 0.1 ml of inoculum into the thighs of mice 2 h before therapy with Antibacterial extracted from bacteria Pseudoalteromonaspiscicida PG01compounds. Two hours after, injection mice were treated with single subcutaneous doses of Antibacterial compounds. The growth of S. aureus inthe control group were determined at four sampling times over 48 h. The treated groups were sampled nine times over 48h. The thighs were removed at each time point and placed in sterile tubes containing sterile phosphate-buffered saline–10% glycerol, and were ground with a homogenizer. Tenfold serial dilutions of each sample were made and plating 0.1 ml from selected dilutions onto staphylococcus-selective media Mannitol salt agar and Muller Hinton agar plates. Plates were incubated for 2 days at 35°C to determine the number of CFU remaining per thigh.

Results: According to the results, bacterial total counts of thigh at 2 h after injection of antimicrobial compounds has begun to decline and 8 h after the injection has been minimal,then, in 24 h after injection, it has started to rise again. In the control group, two hours after the injection, bacterial total count has began increase,and at 24 h after the injection has started to decline. By considering the results, it seems antibacterial compounds extracted from bacteria Pseudoalteromonaspiscicida PG01 , have been effective for reducing the total count of S. aureus.

Conclusion: With the emergence of newer diseases and multi-drug resistant bacteria, it has become essential to develop novel and more effective antibiotics. Many interesting marine natural products with promising pharmacological properties are being developed and will continue to play an important role in studying biochemical events and unraveling their role in cell regulation. Of course, there is greater need for extensive collaborations between chemists and pharmacologists so that these sources meet their full potential and become good candidates for laboratory culture.

Keywords: Pseudoalteromonas piscicida PG-01, Thigh infection model

P445: **Prevalence of MBL enzymes class blaVIM, blaIPM and blaNDM isolates of Pseudomonas aeruginosa isolated from burn wounds in Shahid Sadoughi burn hospital in Yazd**

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Background and Aim: Pseudomonas aeruginosa is a Gram-negative non-fermentative bacteria that plays a major role in development of opportunistic infections and severe infections in burn patients. Produce beta-lactamase enzyme, is a major mechanism of resistance to beta-lactam antibiotics. Occurrence of enzymes capable of inactivating all Metalobetalactams including carbapenem beta-lactam antibiotics(except Monobactams such as Aztronam) is new problem in treatment of patients. The objective of this study was to investigate the prevalence of MBL enzyme blaVIM, blaIPM and blaNDM in Pseudomonas aeruginosa strains isolated from burn wounds in Yazd province.

Methods: Methods: In this cross - sectional study, 180 burn-wound samples were collected from burn patients in burn-hospital belonged to Shahid Sadoughi University of medical sciences in Yazd during one year. Specimens were immediately transferred to Department of Microbiology , Faculty of Medicine and were incubated for 18-24 hours at 37 ° C. Suspected colonies were identified by conventional biochemical methods. Sugar utilization in the OF medium (Oxidation-Fermentation), growth at 42 ° C and pigment production tests were performed for oxidase positive and non-fermentative colonies on TSI.Antibiotic susceptibility was determined by Kirby-Bauer methods according to CLSI standards. ETest MBL method were used for phenotypic detection of MBL and for determination of blaVIM, blaIPM and blaNDM PCR methods using specific primers were used.

Results: RESULTS: At total of 180 isolate 54 P. aeruginosa strains were isolated from burn wounds. Out of 54 isolates, 70% , 66 % and 74% were resistance to ertapenem , meropenem and imipenem respectively and 35(64%), 40 isolates(74%) were resistant tomeropenem and imipenem (MIC> 16 . MBL enzymes were detected in 29.5% isolates. 9(16.6%)and 5(9.2%)of them had blaVIM and blaIPM respectively and 2(3.7%) isolates have blaVIM and blaIPM Simultaneously. None of the isolates had blaNDM

Conclusion: The results of this study shows that the prevalence of MBL enzymes and antibiotic resistance in patients in the burn hospital is high and It is necessary to take Proceedings to control the spread of this nosocomial infections

Keywords: MBL, Pseudomonas aeruginosa, antibiotic resistance

P446: **Evaluation of Salivary and Plaque Streptococcus mutans Counts in children with Acute lymphoblastic leukemia**

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Background and Aim: Dental caries are one of the most prevalent diseases in children with acute lymphoblastic leukemia (ALL). The aim of this study was to determine the caries activity in children undergoing maintenance stage chemotheray.

Methods: Thirty Two leukemic children as study group and 32 normal healthy children as control group selected. The age ranged from 4 to 12 years who were hospitalized under maintenance stage chemotherapy at the Omid Hospital, Esfahan, Iran.The children received only a clinical dental examination. After oral examination, stimulated saliva samples were collected from the subjects to exam the salivary Streptococcus mutans(SM) counts(Dentocult SM Strip mutans). Also plaque samples were collected from 4 surfaces of teeth.Samples incubated for 48 h in 37°c. The results were statistically analyzed using Mann-Whitney Test.

Results: Our study showed that the salivary SM counts in ALL children were significantly lower than control Group ($P<0.001$) Also plaque SM counts in ALL children were significantly lower than control Group($p= 0.001$

Conclusion: Increasing dental decays in ALL children may be associated by another microbial factors such as lactobacilli or anti-cancer therapy.)

Keywords: Streptococcus mutans leukemia saliva plaque

P447: **Salivary Streptococcus mutans counts, salivary pH and dental caries in children with acute lymphoblastic leukemia**

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Background and Aim: Dental caries are among the most prevalent diseases in children with acute lymphoblastic leukemia (ALL). The aim of this study was to evaluate the caries activity in children undergoing maintenance stage chemotherapy courses.

Methods: Thirty Two leukemic children as study group and 32 normal healthy children as control group selected. The age ranged from 4 to 12 years who were hospitalized under maintenance stage chemotherapy at the Omid Hospital, Esfahan, Iran.The children received only a clinical dental examination. Decayed (D), Missing (M), and Filled (F) Tooth surfaces (S) scores were recorded following the WHO criteria. After oral examination, stimulated saliva samples were collected from the subjects to exam the salivary Streptococcus mutans(SM) counts(Dentocult SM Strip mutans)and salivary pH(pH indicating paper). The results were statistically analyzed using t-test.

Results: The Student T-test shows that the salivary SM counts in ALL children were significantly lower than control Group ($P<.05$). Also, the ALL group tended to have lower salivary pH than the control group ($P<.05$). The mean DEFTS/DMFTS scores of the ALL group were higher than the control group($P<.05$).

Conclusion: The decreasing of salivary SM Counts during chemotherapy may be influenced by reduction of salivary volume, the disturbance of the immunological system, or the use of anti-cancer drugs. Increased dental decays seen is due to altered salivary pH in these children and It seems other factors associated with the development of dental caries include poor oral hygiene, carbohydrate-rich diet, sucrose rich pediatric medication, nausea and vomiting (causing acid erosion) and prolonged hospitalization.

Keywords: Streptococcus mutans ,Saliva, acute lymphoblastic leukemia,dental caries

P448: **Antibacterial properties of Peganum harmala seed extract on three bacteria, Escherichia coli, Streptococcus and Staphylococcus**

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Background and Aim: Harmala is shrubs plant, Herbaceous and persistent , owned Zygophyllacea family that it is content the several alkaloids such as Harmaline, harmalol and is harmine

Methods: . In this scheme, the harmala seed extract were added to agar mediums containing Streptococcus bacteria, Escherichia coli and Staphylococcus

Results: The aim of this work was to investigate the antibacterial activities harmala seed Because the seed and other parts of the harmala been used Since ancient times for medicinal purposes

Conclusion: The results indicate that the harmala seed extract on bacteriums Staphylococcus and Streptococcus have positive effect and on the E. coli has had a negative effect

Keywords: harmala, Escherichia coli, Streptococcus ,Staphylococcus

P449: **Molecular detection of Mycoplasma genitalium in infertile women**

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Background and Aim: Mycoplasma genitalium is identified as an etiologic agent of acute and persistent non-gonococcal and non- chlamydial urethritis in women and may play a role in pelvic inflammatory diseases (PID). The development of PID infection can lead to ectopic pregnancy and infertility. PID has a polymicrobial etiology, with Chlamydia trachomatis and/or Neisseria gonorrhoeae that isolated from approximately one-third of case. The aim of this study was to determine the presence of mycoplasma genitalium in female infertile admitted to Mashhad University infertility clinic

Methods: Women with a history of infertility were referred to the Mashhad Medical University infertility clinic. The cervical swab specimens were collected from 100 infertile (as case) and 30 fertile (as control group) women. DNA extraction was performed from clinical specimens using DNA extraction kit. Mycoplasma genitalium was detected by PCR using commercial kit.

Results: The results of PCR reaction showed that the prevalence of M. genitalium was 11% in infertile women and 3.3% in normal fertile women that was statistically significant (p = 0.024). Fifty six of the 100 women had tubal factor infertility (TFI); 10.7% of women with TFI had positive result for M. genitalium, compared with 11.3% of women with normal tubes, that was not statistically significant (p = 0.045).

Conclusion: This study indicates that M. genitalium may be a risk factor in women's infertility, but its association with tubal factor infertility requires further investigation.

Keywords: Mycoplasma Genitalium, infertility, PCR

P450: **A Study on the rodents, fauna and the ZCL reservoir host in Zarqan County, Fars Province, Iran, 2012.**

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Background and Aim: The incidence of zoonotic cutaneous leishmaniasis (ZCL), which is endemic in several parts of Iran, has recently increased in the rural regions around Zarqan city, Fars province. As a part of an investigation of this worrying trend, the study of rodent species has taken place in this city.

Methods: During 2012, wild rodents caught from different parts of this region were caught by sherman traps and checked, by the microscopical examination of liver and spleen smears, for leishmania infection, to see which species were acting as reservoir hosts.

Results: Meriones libycus was the dominant species (80% of 100 rodents collected) that 6.25% of them were found smear- positive for Leishmania amastigote. The other species were Ratus ratus (15%) and Mus musculus (5%), but non of them were found positive.

Conclusion: This confirms M libycus is the probably reservoir host of zoonotic cutaneous leishmaniasis in this part of southern parts of the country.

Keywords: Rodent, ZCL, Zarqan, Iran.

P451: **The study of relation between biofilm formation of Uropathogenic E.coli and antibiotic resistant**

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Background and Aim: Urinary tract infections is the most common nosocomial infections which is caused by colonization of uropathogenic Escherichia coli to host mucosal epithelium and damage host tissue. Cell surface structures of uropathogenic Escherichia coli are involved in biofilm formation. The aim of this study was to determine antibiotic resistance, biofilm formation of uropathogenic Escherichia coli and the relationship between them.

Methods: In this study 50 samples of patients who aere suspected to urinary tract infection (UTI) by E.coli were collected.Antibiotic resistance of uropathogenic Escherichia coli was done by Kirby - Bauer method. Biofilm formation assay was performed by96 microtiter plate.

Results: According to common biochemical tests among of 50 samples, 35 samples were identified as uropathogenic Escherichia coli. 72% of samples showed resistant to Ampicilin and all of them were sensitive to Amikacin. 30,8 and 10 percent of them showed strong ,moderate and week biofilm formation, respectively and 2% had shown no biofilm formation .

Conclusion: Rresults showed that , those E. coli which is caused urinary tract infections and can form biofilm, are resistant to antibiotics too.

Keywords: Urinary tract infections, Uropathogenic Escherichia coli, Biofilm formation

P452: **Immunological Evaluation of Salmonella Typhimurium OPS Conjugate to Diphtheria Toxoid in Mouse as a Vaccine Candidate**

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Background and Aim: Salmonella typhimurium not only can cause gastroenteritis in humans but also it can cause paratyphoid in some animals. Still produce a vaccine for the disease is not complete. Purpose of this study was to evaluate the immunogenicity of diphtheria toxoid conjugate OPS with Salmonella typhimurium in mice as a candidate vaccine.

Methods: OPS of this bacteria extracted by hot phenol method, then dialysis and electerophoresis were done.To improve immunogenicity, the purified antigen was coupled to DT with ADH as a spacer and EDAC as a linker. The reaction mixture was passed through a sepharose CL-2B column. Sterility and toxicity tests were shown that prepared conjugate was non-toxic and non-pyrogenic.Then four group of female BALB/c mice (each group was included 15 mice) was selected. Vaccination was performed by three doses with two week interval. Then serum samples was collected and antibody responses was measured by ELISA method for total IgG, IgG1, IgG2a, IgG2b, IgG3, IgM and IgA.

Results: Two week after first dose of vaccination there was no significance different antibody titers between groups that were immunizided by OPS and OPS-DT. But after second and third doses, OPS-DT showed significance increasing in all types of antibodies titers in versus OPS. Overall results of anti OPS inductions for total IgG, IgM, IgA, IgG1, IgG2a, IgG2b, and IgG3 were shown: OPS-DT>OPS >DT. The anti OPS IgG antibody was predominantly of IgG1 subtype and then IgG3, IgG2a, IgG2b, respectively.

Conclusion: These results indicated that OPS Salmonella typhimurium increase anti OPS antibodies in conjugate form with diphtheria toxoid and can be an appropriate effective candidate vaccine for this bacteria.

Keywords:: Salmonella typhimurium, OPS, DT, conjugate, ELISA

P453: **Determination of Resistance Pattern of Isolated Acinetobacter baumannii from Hospitalized Patients in Imam Reza Hospital, Tabriz**

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Background and Aim: Acinetobacter baumannii is an opportunistic pathogen which play the more and more greater role in the pathogenicity of the human. It is often attached with the hospital environment, in which is able easily to survive for many days even in adverse conditions. These bacteria are a leading cause of therapeutic resistant nosocomial infections especially in hospitalized patients in intensive care Units. The aim of this study was to isolate the Acinetobacter baumannii species from hospitalized patients and to determine antimicrobial resistance pattern of these bacteria for selection of appropriate antibiotics.

Methods: Samples were collected from patients and transferred to the laboratory under standard conditions. Bacteria were isolated and purified by conventional culture methods. Identification of bacterial species was performed by standard biochemical tests. The isolates that were identified as Acinetobacter baumannii were subsequently tested for antibiotic resistance by the disk diffusion agar method for 11 different antibiotics. The tests were carried out on Muller Hinton agar (MHA) plates and incubated at 35˚C for 18 hrs.

Results: Out of the 40 clinical Acinetobacter baumannii isolates collected, 38 (95%) were multi drug resistant (MDR). To determine the Disk diffusion agar method, the highest levels of antibiotic resistance were seen against Imipenem, ceftazidime, ceftriaxon, and ciprofloxacin (98%). Amikacin and Tobramycin (50%) were the most effective antibiotics against Acinetobacter baumannii.

Conclusion: Our results confirm the high prevalence of Acinetobacter baumannii resistant isolates and the ensuing therapeutic problems in Iran. Determination of the resistance patterns of these bacteria according to MIC is necessary, and it can be especially helpful in treatment of haspitalized patients.

Keywords: Acinetobacter baumannii, Antibiotic resistant, Minimum inhibition concentration

P454: **Pseudomonas aeruginosa-a serious risk in Zare hospital,Sari,Iran**

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Background and Aim: One of the major opportunistic pathogens in patients with burn injuries is Pseudomonas aeruginosa, which causes severe infections in burned patients.The objective of this study was to investigate the antimicrobial susceptibility pattern of Pseudomonas aeruginosa isolated from burn patients.

Methods: Atotal of 143 isolates of Pseudomonas aeruginosa were obtained from burn wound of 250 patients in Zare hospital in Sari,Iran.Antimicrobial susceptibility to 9 antimicrobial agents was determined by disc agar diffusion test.

Results: The rates of resistance to different antibiotics were as follows: Amikacin %60.13, Gentamicin %59.44, Ceftazidime %53.14, Ceftriaxone %61.53, Piperacillin %58.74, Imipenem %60.83, Meropenem %65.73, Ciprofloxacin %58.74, Cefepime %60.83, and 70(%48.95) isolates were resistant to all of the antibiotics

Conclusion: The high prevalence of resistance in Pseudomonas aeruginosa isolated in burn patients confirm that proper infection control practices and barriers are essential to prevent spreading and outbreaks of resistant strains of Pseudomonas aeruginosa in Zare hospital.

Keywords: Pseudomonas aeruginosa,Burn patients,Antibiotics,Resistance

P455: **Phenotypic and Genotypic Detection of community-acquired CTX-M producing klebsiella pneumoniae in sanandaj, iran.**

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Background and Aim: Organisms producing CTX-M β-lactamases are emerging as a source of resistance to oxyiminocephalosporins such as ceftriaxone and ceftazidime. However, the laboratory detection of these strains is not well defined. In this study, phenotypic assay for screening of extended-spectrum β-lactamases producing strains and molecular assay for the identification of CTX-M β-lactamases genes was developed and used to investigate the prevalence of these enzymes among community-acquired Klebsiella pneumoniae in sanandaj, Iran.

Methods: In this analytic-descriptive study, 100 klebsiella pneumonia strains community-acquired were used. The pattern of antimicrobial resistance was determined by disk diffusion (double-disk test) method. The ESBL production was determined by combination disk method using disks containing ceftazidim and cefotaxim alone and in combination with Clavulanic acid. CTX-M type of ESBL producing genes was detected by PCR.

Results: Confirmatory phenotypic test showed that 88% of the strains were ESBL positive. PCR used for the detection of CTX-M gene, showed that 37(42.04%) out of 88 isolates contained such gene.

Conclusion: Noticing the increasing rate of the ESBLs producing strains, using the appropriate treatment protocol based on the antibiogram pattern of the strains is highly recommended.

Keywords:: CTX-M gens, klebsiella pneumonia, Extended-Spectrum β- Lactamases, community-acquired, PCR

P456: **Quantitative analysis of Phospholipase B(PLB1, PLB2) gene expression Candida albicans resistance and sensitive of fluconazole isolated from Cancer patients suffering oral Candidiosis in Tonek**

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Background and Aim: Oral candidiasis is a fungal infection which caused by opportunistic Candida species, especially Candida albicans and in certain individuals, particularly those undergoing chemotherapy is very common and dangerous. Recently, with the development of drug resistance of oral candidiasis toward antifungal drugs such as fluconazole, the need for identifying genes effectiveness in this regard is felt more than ever. Due to the structural role of phospholipase infection and pathogenesis in Candida albicans, this study compared a phospholipase B (PLB1, PLB2) gene expression in susceptible and resistant strains of Candida albicans in oral candidiasis of patients with cancer

Methods: 70 mouth swab samples of patients with oral candidiasis cancer were collected. After culturing on sabouraud dextrose agar medium, containing chloramphenicol and cycloheximide through mycological methods (germ tube test, chlamydospore test, Krumagar and APA test) Candida albicans was identified. Isolating sensitive and resistant samples was done through drug susceptibility testing by Microdilution method according to NCCLm27a instructions. Then the RNA was extracted from several resistant and sensitive samples and comparison one step RT-Real Time PCR reaction with phospholipase gene-specific primers (PLB1, PLB2) B was administered

Results: our studies on 56 susceptible and resistant samples showed that selected conditions the expression of PLB1 gene in resistant samples was considerably more than sensitive samples but no significant difference in PLB2 gene expression was observed in susceptible and resistant samples.

Conclusion: PLB1 gene can be considered as one of the factors affecting the resistance of Candida albicans and should be considered as the next target for therapeutic approaches.

Keywords: Candida albicans , Oral candidiasis , Phospholipase B(PLB1, PLB2)

P457: **Investigation of antibacterial effect of native Mentha pulegium extract on Streptococcus mutans in vitro in Meshkinshahr, Iran**

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Background and Aim: Most of the populations in the different areas of world are affected by bacterial infectious of dental caries and the high cost of cure, especially in high-risk groups such as people with dry mouth, prevention and control is vital. However, due to the popularity and importance of international community to treat traditional medicines derived from natural ingredients and herbs, the present study investigated the effect of local antibacterial Mentha pulegium extract from Meshkinshahr, Iran, on Streptococcus mutans, bacteria that cause tooth decay, were used in vitro.

Methods: The composition of extract of this medicinal plant, obtained from leaves, was extracted by maceration method was analyzed by GC and GC/MS system, and their antibacterial effects on S. mutans were determined by broth macrodilution test method. The results were analyzed with Mann Whitney test ($P<0.05$).

Results: The extract is particularly rich in Pulegone (31.78%), 1, 8-cineole (15.99%), Menthoforan (11.25%), Cis- Isopulegon (10.5%) and Paramenth-3- n 8-l (6.85%). The results of broth macrodilution, rates of MIC and MBC (µg/ml), and the level of significance ($P <0.05$) were shown for native pennyroyal extract on studied bacteria were 11.9 and 98 µg/ml respectively.

Conclusion: Present study showed the compositions of native pennyroyal extract had growth inhibitory effect on S. mutans ($P <0.05$) and also had bactericidal effect on the bacteria in the present concentration range.

Keywords: Antibacterial activity, Mentha pulegium, Streptococcus mutans, Meshkinshahr, Iran

P458: **The comparison of the anti-microbial properties of silver,titanium,and copper nanoparticles against Multidrug-resistant pseudomonas aeruginosa and their cytotoxicity effects on L 929 cell line**

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Background and Aim: There is a growing concern regarding the emergence of drug-resistant pathogens such as multidrug-resistant bacterial strains,therefore, the development of newer antimicrobial compounds is of high research priority. Recently metallic nanoparticles have gained importance because of their anti- microbial activities. The present work includes an analysis of anti bacterial effect of silver,titanium di oxide and copper oxide nanoparticles against Pseudomonas aeroginosa(MDR). The toxicity assessments of the nanoparticles against the fiboblast cell line (L929)were determined as well.

Methods: Minimum inhibitory concentration (MIC)and minimum bactericidal concentration(MBC)of three metal nano particles were determined on Pseudomonas aeroginosa(MDR). Cytotoxicity effects of the nanoparticles were assessed by MTTassay.

Results: The complete antibacterial activity of the three nanoparticles was as low as 64 micro g/ml for silver nanoparticles and 512 micro g/ml for titanium di oxide and 256 micro g/ml for copper oxide.These nanoparticles showed toxic effect on fibroblastcell line (L929).

Conclusion: The results indicated that metallic nanoparticles are effective bactericidal agents that are not hindered by the drug resistant mechanisms of clinical relevant bacterium but these nanoparticles affected L929cell viability while inhibiting considerable percentage of MDR P.aeroginosa.

Keywords: MultiDrug-Resistant Pseudomonas aeroginosa,Nanoparticles,toxicity.

P459: **Evaluation of antibiotic resistance klebsiella isolated from urinary tract infections by the routine methods and PCR**

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Background and Aim: Numerous use of Beta Lactamase in treatment of bacterial infections led to drug resistance of such bacteria. One of difficulties in treatment of hospital infections is Extended Spectrum β Lactamase (ESBL) among isolated strains of Klebsiella pneumoniae. Since some of ESBL strains shows double reaction in drug sensitivity test at in vitro and in vivo condition, it makes difficulties in selection of sufficient treatment. This study aimed to evaluate antibiotic susceptibility pattern of betalactam antibiotics and reaserch on β lactamase gene including blaSHV, blaTEM, blaCTX-M in K.pneumoniae clinical isolates.

Methods: In the present study 40 urine samples collected from Shahroud Khatamolanbia Hospital were studied. Out of the 40 samples, 20 K.penumoniae isolates were detected by standard biochemical tests. Susceptibility to antimicrobial agents was tested for 13 antibiotics. ESBL production was screened by Double Disk Test. Finally, the screened isolates were investigated by PCR assay to detect the special isolated that are responsible for production of enzymes including SHV, TEM, CTX-M.

Results: In this study, the maximum amount of resistance to nitrofurantoin resistant strains were found at 15 isolates (75%). The maximum sensitivity to imipenem was observed in the 12 isolates (60%). Morover, ten isolates (50%) of the study contained ESBL genes. Also, seven isolates (70%) contained the blaSHV gene and five isolates (50%) contained the blaTEM gene. Besides, three isolates (30%) contained blaCTX-M gene. Also, one isolate (10%) contained both of blaSHV and blaTEM genes simultaneously. The simultaneous existence of ? lactamase, was observed in none of the isolates.

Conclusion: Due to the high percentage of resistance against third-generation cephalosporins, accurate antibiogram tests before prescribing the antibiotics in infection caused by organisms that produce ESBL, are an inevitable necessity.

Keywords: Klebsiella pnuemoniae, ESBLS, Urine infection, blaSHV, blaTEM, blaCTX-M, PCR.

P460: **Bacteriophage: A tool to biofilm inhibition and remove**

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Background and Aim: Pseudomonas aeruginosa is an ubiquitous organism which has emerged as a major threat in the hospital environment. Overuse of antibiotics has also significantly increased the emergence of antimicrobial multiresistant bacteria. P. aeruginosa has an innate ability to adhere to surfaces and form virulent biofilms. Bacteriophage might be represented as one attractive solution to this problem. In this study, P.aeruginosa phage were utilized to Biofilm inhibition and remove.

Methods: Sample collected from University sewage and then isolation was done according to Martha.R.J.Clokie protocol. Serial dilution prepared, and equally incubated to bacteria for investigating of Biofilm inhibition potential. Biofilm formed based on Microplate Biofilm Assay. The effect of isolated phage investigated on biofilm remove of Pseudomonas putida, E.coli and Acinetobacter baumanii.

Results: P.aeruginosa biofilm had OD: 1.688 in 492n.m. Pure phage, 10-2 and 10-3 diluted phage decreased OD to 1.587, 1.341 and 1.461, respectively. Isolated phage dramatically decline OD of Biofilm of all strains. P.putida formed strong biofilm by OD: 0.221 in 492n.m but dramatically declines after of phage recruitment. Manipulated phages in serial dilution 10-1, 10-2, 10-3 decrease OD to 0.078, 0.062, 0.067, respectively. There was same result for two other bacteria.

Conclusion: According to our knowledge, phages have potency to inhibit biofilm formation and also they could be exploited to remove biofilm. Our results shown phages have various affinity to attach to hosts, thereby it is supposed to phages compete for their receptors. Therefore it is supposed phages have most efficiency in optimum concentration to remove biofilm or growth inhibition.

Keywords: Bacteriophage, Biofilm, Planktonic, Biofilm remove and Biofilm inhibition.

P461: **Consideration and Comparison of Antibacterial Susceptibility for Bacteria Causing Peritonitis**

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Background and Aim: The peritonitis is a dangerous disease with mortality rate about 10% that in moderate one should be treated with antibiotics instead of surgical operation. Therefor, diagnosis of the type and percentage of the bacteria causing peritonitis and to determine the effective antibiotics to avoid of surgical operation or appropriate and effective treatment of patients (after operation) is essential.

Methods: This research is a descriptive – analytical study that was conducted on 100 patients after peritonitis surgery to isolation and identification the bacteria causing peritonitis from patients' peritoneal fluid to determine 20 kind of antibacterial susceptibility by Kirby-Bauer disk diffusion and agar serial dilution methods for determining of MIC.

Results: The frequency distribution tables, diagrams, chi-square, t-test and one way anova were used to describe and analyze the data. In this research 8 bacteria causing peritonitis are being identified which were sensitive to Tobramycin, Ceftizoxime (MIC = 4µg/ml), Amikacin(> 90%) and Cefotaxime, Ciprofloxacin (MIC = 0.5µg/ml), Ceftriaxone, Cefixime (80-90%). Significant statistical relationship was observed between patients' sex and etiology of peritonitis ($P < 0.05$). No significant statistical relationship was observed among sex, age, smoking and obesity of patients with antibacterial susceptibility (20 types). ($P > 0.05$)

Conclusion: With respect to the above mentioned results, the most prevalent bacterium causing peritonitis was E.coli (66%) and using of Tobramycin, Ceftizoxime, Amikacin, Cefotaxime, Ciprofloxacin, Ceftriaxone and Cefixime are most effective and is recommended in treatment of moderate peritonitis to avoid the surgical operation or effective treatment after surgery for any type of peritonitis without looking at sex, age, smoking or obesity.

Keywords: Peritonitis, Antibacterial Susceptibility, Bacteria

P462: **The relation between efflux pump gene carriage and biocide resistance**

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Background and Aim: Although, antiseptics are most widely used as anti bacterial agents in hospitals. There is a little information on reduced susceptibility to these biocides. Furthermore, relation between the presence of antiseptic resistance genes and reduced susceptibility to these biocides is less investigated. The quaternary ammonium compounds (QACs) that are widely used as disinfectant contain NH4+. The labels often list a form of ammonium chloride (AC) such as benzyl, didecyl, dimethyl, octyl or a combination of them. Resistance to QACs is widespread among a diverse range of microorganisms and is facilitated by several mechanisms such as modifications in the membrane composition and expression of efflux pump genes.

Methods: Eighty five K. pneumoniae strains were isolated from different clinical samples and susceptibility to Deconex was assessed using micro-broth dilution method. Sterile 96-well micro plates were used for the assay. Serial dilution of Deconex while inoculated with the bacterial suspension was done and incubated at 37 oC overnight. The minimum inhibitory concentration (MIC) values for all strains were determined. The presence of the qacdeltaE gene was also identified by polymerase chain reaction.

Results: Results: Biocide susceptibility, tested by micro-broth dilution method, showed that 52 strains had reduced susceptibility to QAC and in 18.8% (16) of strains antiseptic resistance gene, qacdeltaE were found. Among all strains 29.4% (25) possessed antiseptic resistance genes.

Conclusion: In this study the relationship between carrying of efflux pump gene, qacdeltaE gene and reduced biocide susceptibility in K. pneumoniae clinical isolates was showed.

Keywords: QAC, qacdeltaE, susceptibility, K. pneumoniae

P463: **Genetic Characterization of a Vancomycin-Resistant Staphylococcus aureus Isolate from the Patient in the Northeastern Iran**

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Background and Aim: Emergence of vancomycin-intermediate Staphylococcus aureus (VISA) and vancomycin-resistant S. aureus (VRSA) strains has led to global concerns about treatments for staphylococcal infections. These strains are currently rare even though there is an upward trend in their reported incidence. Therefore, appropriate screening and epidemiological evaluation of VRSA strains can affect future global health care policies.

Methods: Isolates of Staphylococcus aureus were obtained from various clinical samples and were then evaluated with agar screening, disk diffusion, and MIC methods to determine resistance to vancomycin and methicillin. After confirmation of the isolated VRSA strain, genetic analysis was performed by evaluating mecA and vanA gene presence, SCCmec, agr, and spa types, and toxin profiles. Multilocus sequence typing (MLST) and plasmid analysis were also performed.

Results: The VRSA strain was resistant to oxacillin (MIC of 128 g/ml) and vancomycin (MIC of 512 g/ml). Disk diffusion antimicrobial susceptibility tests showed resistance to oxacillin, vancomycin, levofloxacin, ciprofloxacin, trimethoprim-sulfamethoxazole, clindamycin, rifampin, and tetracycline. The isolate was susceptible to minocycline and gentamicin. PCRs were positive for the mecA and vanA genes. Other genetic characteristics include SCCmec type III, agr I, spa type t037, and sequence type (ST) 1283. The plasmid profile shows five plasmids with a size of about 1.7 kb to>10 kb.

Conclusion: The isolated VRSA strain was obtained from a critically ill hospitalized patient. Genetic analysis of this strain suggested that the strain was a methicillin-resistant S. aureus (MRSA) clone endemic in Asia that underwent some genetic changes, such as mutation in the gmk gene and acquisition of the vanA gene.

Keywords: Staphylococcus aureus, vancomycin, resistant, PCR, Molecular typing

P464: **Assessment of minimum vaccination coverage of Measles in Iran by using the time series SIR model**

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Background and Aim: Measles is one of the most contagious diseases in infancy. Recently, computational modeling including Susceptible-Infective-Resistance (SIR) has an important role in epidemiological studies. This model is based on mathematical answers for questions related to existence or absence of epidemic elements. This study is aimed to determine the existence of infections resulted from Measles and to assess its minimum vaccination coverage by recruiting SIR model through 1977-2010 in Iran.

Methods: Data were recruited from Iran Center of Disease Control (Health Ministry of Iran) and R0 and P parameters were analyzed by using the SIR model during 1977-2010.

Results: Based on R0 results, there is an epidemic condition for Measles in Iran. Vaccination coverage of Measles is going to be in an inappropriate situation year by year. P parameter was estimated for 2008 and 2011 years 9.671543 and 10.08324, respectively.

Conclusion: Our findings indicate that current vaccination coverage in Iran is not suitable. Since, it is suggested that the policy of Health Ministry should be improved to achieve a sufficient Measles vaccination coverage.

Keywords: SIR model, Measles vaccination, Minimum coverage, Iran.

P465: **Prevalence investigation of Staphylococcus aureus and Staphylococcus saprophyticus among ICU, Men and Children wards in Imam Khomayni Hospital, Ilam, 2011-2012**

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Background and Aim: Staphylococcus aureus is a skin and nasal flora normal and known as a main strain of Staphylococcus species which cause various diseases such as Impetigo, Toxic Shock Syndrome, scalded skin syndrome, and etc. Staphylococcus saprophyticus is a coagulase negative and a common agent of urinary tract infections. Hospital acquired infection as an old challenge has high importance in hospital infection control and Staphylococcus spp. play main role among routine pathogens. In other hands, antibiotic resistance emergence among Staphylococcus spp. specially coagulase negative species contribute to hospital acquired infection therefore this study is designed to investigate the prevalence of Staphylococcus aureus and Staphylococcus saprophyticus among ICU, Men and Children wards.

Methods: Samples collected randomly from ICU, Men and Children wards. Through 203 sampling of wall, floor, bed, pillow and blanket, 75 Staphylococcus spp. isolated. Species were recognized base on culture on Mannitol salt agar and Novobiocin susceptibility determination.

Results: Among 75 samples 62 isolates were Staphylococcus saprophyticus, and 13 ones were Staphylococcus aureus. 51% of bacteria isolated from ICU, 29% from children ward and 20% from Men surgery ward. Staphylococcus saprophyticus was comprised 87%, 82% and 73% of ICU, Children ward and Men surgery ward isolates, in a row.

Conclusion: Our funding indicates that there is an inappropriate instruments to deal with infection in this hospital, especially in ICU. Regards to this issue that Staphylococcus spp. as a main pathogen which has potency to form biofilm and show high resistance to extended broad antibiotics therefore it is suggested to prepare appropriate guideline to cope with bacteria dissemination and resistance emergence in hospital.

Keywords: Staphylococcus saprophyticus, Staphylococcus aureus, ICU, Children ward and Hospital infection

P466: **Prevalence of Toxoplasmosis and its association with age, education and region of residency among women whom referred to check up TORCH syndrome during 2011-2012 at Hamadan, Iran**

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Background and Aim: Toxoplasmosis is a parasitic disease caused by the protozoan Toxoplasma gondii.[1] The parasite infects most genera of warm-blooded animals, including humans, but the primary host is the felid (cat) family. Up to a third of the world's human population is estimated to carry a Toxoplasma infection. CDC notes the overall sero-prevalence in the United States as determined with specimens collected between 1999 and 2004 was found to be 10.8%, with sero-prevalence among women of childbearing age (15 to 44 years) 11%. Current study is aimed to investigate the prevalence of toxoplasmosis among young women who referred to check up for TORCH syndrome at Hamadan during 2011-2012.

Methods: Data of 2523 women collected base on researcher designed essay. Demographic criteria involved in Age, Education and Region of residency. Antibodies serum level were examined by ELISA. IgG titration equals and more than 1/200 was presumed as sero-positive. Statistic data analyzed by SPSS20 (ANOVA Pack).

Results: 26.1% of IgG sero-positive persons were City residents while 32.3% of them lived at village and suburb of city. 1.4% and 1.1% of at risk person (base on IgG titration) were city and village residents. 1.3% and 1.9% of IgM sero-positives were city and village residents. The percentage of at risk persons of city and village (base on IgM titration) were 0.3% and 0.6%, in a row. 29.7% of IgG sero-positives did not have academic education while 27.6% of them graduated from high school, at least. The sero-positive IgM percentage of non-academic educated persons and graduated/academic ones were 1.7% and 1.4%.

Conclusion: Our funding indicate the association between age of women and their level of education with percentage of contamination and prevalence. Also, here is a relationship between of residency region and toxoplasmosis occurrence. IgG sero-positive percent (26.9%) is near to US sero-positive (22.5%.) percentage. IgM sero-positive (2%) is more lesser than IgG sero-positive (26.9%) that illustrated the toxoplasmosis is chronic or there is previous contact. Unfortunately, there is some restriction in this study such as no information about women job. Therefore, because job is one of the main criteria in toxoplasmosis study, it is suggested there is need a more study about the association between job and toxoplasmosis.

Keywords: Toxoplasmosis, Pregnant women, Sero-positive, age, education and region of residency

P467: **Frequency investigation of HCV and HBV among addicted persons through 2011-2013**

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Background and Aim: Hepatitis known as a huge health issue throughout the world and a global challengeable disease. HCV and HBV could transmit via blood, sexual and parental ways and also HAV and HEV have oral-fecal transmission. Therefore, Hepatitis could disseminate in society easily. Addicted persons introduced as a human source who could transmit Hepatitis via common syringe usage. This study is designed to investigate the prevalence of HCV and HBV among addicted person during last 2 years in Iran.

Methods: Papers searched on viable data banks such as Pubmed, Sciences Direct, Magiran, and SID base on related keywords. Related papers were excluded and included base on PRISMA diagram 2009. 19 papers were entitled HCV and HBV among addicted persons that published during last 2 years. These papers include 3700 of addicted persons who had hepatitis, simultaneously.

Results: 96% of ones were men (3563) and 4% were women. HBV, HCV and HCV-HBV(at the same time) were 42%, 5% and 5%, respectively. 47% of recruited diagnostic methods were ELISA. ELISA+PCR, PCR, ELISA+ Riba and PCR+Riba were 26%, 16%, 5% and 5%, in that order. 52.6% of studied population did not have any identified age details while 26.3% of them were between 20 till 40 ages. Upper 40 years ones and also persons with the range between 1- 20 ages were 10.5% and 10.5%, in a row. Most of addicted persons were belonged to zone 1 (Tehran and Suburbs: 36.8%). The percentage of addicted ones who lived in zone 5 (Isfahan, Yazd and Kerman) and zone 9 (other cities) were 26.3% and 15.7%.

Conclusion: Our funding indicate most of addicted population were men who were between 20-40 years old, and HBV is most common than HCV. Mega city of Iran like Tehran, Ishfahan, Kerman and Yazd have high prevalence of Hepatitis among addicted persons. ELISA is routine methods around Iran although some of Clinical centers and Laboratories prefer to use specific molecular methods like Riba and PCR. It is presumed that Health Ministry's policy could be improved to deal with issue among addicted persons. Free syringe dispatch among these population could be control Hepatitis transmission in society. And also, there is a need supportive policy to prepare specific methods to screening Hepatitis in addicted population that might leads to quarantine Hepatitis positive addicted persons from society.

Keywords: HCV, HBV, Addicted persons, Iran

P468: **Frequency investigation of HCV and HBV among addicted persons through 2001-2011**

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Background and Aim: Hepatitis known as a huge health issue throughout the world and a global challengeable disease. HCV and HBV could transmit via blood, sexual and parental ways and also HAV and HEV have oral-fecal transmission. Therefore, Hepatitis could disseminate in society easily. Addicted persons introduced as a human source who could transmit Hepatitis via common syringe consumption. This study is aimed to explore the prevalence of HCV and HBV among addicted person during 10 years in Iran.

Methods: 195 papers searched on viable data banks such as Pubmed, Sciences Direct, Magiran, and SID base on related keywords. Related papers were excluded and included based on PRISMA diagram 2009. 35 papers were entitled HCV and HBV among addicted persons, published through 10 years. These papers include 348,353 of addicted persons who had hepatitis, concurrently.

Results: 56% of addicted persons had no identified age details. 20-40 ages group were 12% while upper 40 ages group were 9%. 100% of studied group were drug users (Drug injecting user) and 59% of them had AIDS. HBV and HCV were 59% and 29%, respectively. HCV-HBV co-infection were 9%, and HCV-HIV co-infection like HBV-HIV co-infection were 6%. Zone 8 (Hormozgan, Fars, Khozestan) had high frequency (29%), while zone 1(Tehran and Suburbs) and zone 5 (Isfahan, Yazd) were 14%.

Conclusion: Our funding shown most of drug injecting users which have Hepatitis were 20-40 ages. 59% of them were HBV positive and the percentage of AIDS positive persons were 59%. Zone 8 had high frequency by 29% and zone 3, 4, 6 had less frequency. HBV and HCV had same co-infection frequency by HIV and HBV-HCV co-infection were more frequent. It is presumed that the common syringe usage play a main role via hepatitis and HIV transmission among drug injecting user, therefore Health Ministry policy about free syringe dispatch among this group could be an effective way to cope with hepatitis and other blood transmission diseases in society.

Keywords: HCV, HBV, Addicted persons, Iran

P469: **Phage Antibiotic Synergysm(PAS) inhibitoty effect on P.aeroginosa growth in invtro condition**

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Background and Aim: Bacteriophage is a kind of virus that could infect bacteria. Lytic phages are known as a candidate for therapeutic purposes, because they quickly reproduce within and lyse the bacteria in their host range, growing exponentially in number in their process. Nowadays, the rise of antibiotic resistance infections and on the other hand inefficiency of antibiotics to control of diseases , make phages as an attractive field of study to overcome the problems related to bacteria. Combination of two or more antibacterial agents could be an option to remove or treat bacterial infection which are resistant to treatment. Therefore, we designed this study to investigate combined effects of phages and antibiotics on P.aeroginosa against their effects solely.

Methods: Sample were collected from university sewage. Isolation was done according to Martha. R. J. Clokie protocol. Two clinical and ATCC strains of P.aeroginosa were recruited and phage plaque assay was done by spot method. Cefoxitin, Cefotaxime, Clindamycin, Erythromycin, Gentamicin, Neomycin, Chloramphenicol, Amoxicillin, Amoxil-clave, Trimetoprim were exploited to evaluate P.aeroginosa susceptibility and PAS effect.

Results: Although, both strains were resistance to all of the antibiotics except Neomycin and Neomycin (Just ATCC strain), they shown susceptibility to isolated phages. Obviously, PAS effect was seen for Gentamicin, but it was weak for Neomycin.

Conclusion: Our funding indicate phages could be suitable antibacterial agents to inhibit P.aeroginosa growth. Not only phages are effective in resistant cases but also they improve antibiotcs efficiency. Thereby, phages as synergistic agents could be used to treat resistant bacterial infections. This study is on progress, and we tried to investigate PAS effect of phages by MIC and MBC methods.

Keywords: Phage, PAS, P.aeroginosa, invitro, sewage.

P470: **Analysis of synonymous codon by usage of bias and amino acid composition in Hepatitis C Virus**

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Background and Aim: Studies of codon usage pattern can reveal the molecular evolution of organisms, and contribute to understand the interaction between RNA viruses and the immune response of the host. Hepatitis C virus (HCV), as a small enveloped RNA virus, causes chronic hepatitis. It consists of a single-stranded positive-sense RNA by almost 9.6 kb genome, which contains an open reading frame (ORF) that encoding a polyprotein precursor by approximately 3000 residues, flanked by untranslated regions (UTR) at both ends. HCV is classified into at least seven major genotypes that differs in their nucleotide sequences by 31–34% .These genotypes (1-7) show differences with regard to their worldwide distribution, transmission and disease progression. Because of various genotypes shown different drug resistance, at this study we survey the differences and similarities of codon between different genotypes. In other words, it must also be used to genotype the maximum and minimum resistance types.

Methods: 136 Open Reading Frame (ORF) sequences selected among seven genotypes and sub-genotypes of HCV, from NCBI (http: //www.ncbi.nlm.nih.gov) and los Alamos (http: //hcv.lanl.gov). Relative Synonymous Codon Usage (RSCU) values of each codon (for all 136 complete coding sequences of HCV) were calculated according website Gene infinity (http: //www.geneinfinity.org/sms/sms_codonusage.html.) Values >1.0 have positive codon usage bias (abundant codons), while those with RSCU values <1.0 have negative codon usage bias (less-abundant codons), and when the RSCU values is 1.0, it means that these codons are selected equally or randomly. For statistical analysis we use Minitab.16 software. Finally, dendrogram of similarities and differences made with Minitab software.

Results: Cluster analysis shown that codon preference for G or C nucleotides at the end of all amino acids was. For example; Alanine, Glycine, Valine, Tyrosine that were with four codon, Terminal nucleotide of codon preference is G or C nucleotide. Based on the study of codon preferences, genotype 1, 3, 5 and 7 are at the first branch and genotypes 2, 4 and 6 are at the different branch. Genotype 7 is more different than others in the first category thus genotype 7 might differ than other genotypes during the evolutionary process.

Conclusion: Taken together, our analysis revealed that the mutational pressure was the central factor accounting for the codon usage pattern, and the selection pressure also accounted for HCV codon usage pattern. Classification of HCV sequences by using codon usage pattern as a parameter is a better resolution for comparing the homology based approaches. Results reveal that there is much codon usage bias in HCV genome at the different genotypes. In the future studies, analysis of diverse factors that can effect on codon usage variation, can growth our knowledge about processes involved in the HCV evolution and the selective powers that significantly influence codon usage bias.

Keywords: ORF, UTR, HCV, RSCU, NCBI, Analysis of synonymous codon

P471: **The study of antibiotic resistance genes isolated in E. coli in urine tract infections in outpatients in Tehran, Iran**

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Background and Aim: Escherichia coli is the most prevalent agent of urinary tract infection in both outpatients and hospitalized patients. In recent years, due to extended-spectrum β-lactamases (ESBLs), cases of resistance to antimicrobial agents especially to cephalosporin has raised considerably. This study was aimed to determine the survey of CTX-M ،SHV ،TEM ،OXA-1 ،PER-2 و VEB-1 in E. coli isolated from outpatients with UTI at selected laboratories in Tehran, Iran.

Methods: this study that we had done during 6 months, from the first of October to the end of March. After isolation test, we identified 123 samples (13.63%) from among of 1677 urine samples as E. coli and then by using double disk method, 56 samples (45.52%) have recognized as ESBL and we done PCR method on this samples in order to examine studying genes after gene extraction with special kit primarily by using universal PCR and for being sure of accurate gene extraction.

Results: Among 1677 urine samples, 13.63% are E. coli and after double disk method, 45.52% are positive according to ESBL Molecular PCR also shows 51.8% CTX-M, 7.1% TEM, 3.6% SHV, 42% OXA-1, 8.9% PER-2 but we didn't find VEB-1.

Conclusion: Because of susceptibility of genes, the curing of infection causing by this bacteria fails despite of the existence of convenient recognition ways. According to statistical view, there is a direct relation between gender and age in this illness, so we can say that there is a direct relation between the ability in ESBL production and the existence of examining genes.

Keywords: ESBL, E. coli, CTX-M, OXA-1, Doubble disk, Universal PCR

P472: **Dissemination of CTX-M-type β-lactamases among E.Coli isolated from urinary tract infections in Rasht, Iran**

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Background and Aim: The rapid spread of resistance to broad spectrum of β-lactam antibiotics in pathogenic strains of bacteria has recently become health problem in the world. One important mechanism of resistance in gram negative bacteria against β-lactam antibiotics is production of beta-lactamase enzymes. The most important beta-lactamase are Extended-spectrum β-lactamases (ESBLs) which are enzymes that mediate resistance to extended-spectrum cephalosporins (ESCs), such as cefotaxime, ceftriaxone, ceftazidime and the monobactam aztreonam. In recent years a new family of plasmid mediated ESBL called CTX-M has been identified and was named CTX-M type beta-lactamases owing to their high activity against cefotaxime.

Methods: . In this study, thirty three E. coli strains were isolated from urine patients with urinary tract infection. Double-disk synergy test (DDST) was performed for all isolates for screening ESBL. For determining of CTX-M types of ESBL, PCR amplification was used by specific primers related to subtype groups (1 and 2).

Results: Our finding showed that 24% of isolates (8 strains) were detected as ESBLs. PCR analysis revealed that most of the isolates had one kind of CTX-M-1 or CTX-M-2 or both of them.

Conclusion: Because of the possible horizontal transfer of these resistance genes to other pathogenic bacteria, monitoring and control of these resistance strains is very important.

Keywords: Resistance, ESBL, CTX-M, DDST, β-lactam

P473: **Prevalence of serum anti Cytomegalovirus (CMV) virus antibodies among pregnant women in Kashan**

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Background and Aim: CMV is a contagious viral disease with few complications except in pregnant women. CMV infection in pregnancy can result congenital CMV. Effective vaccination programmes are critical to the elimination of CMV infection. The aim of this study was to determine the prevalence of anti-CMV virus antibodies among pregnant women referred to Kashan Central laboratory.

Methods: A cross-sectional study of seroprevalence study was conducted between Feburary 1, 2009, and Feburary 5, 2012.348 women referred in Kashan Central laboratory, were enrolled in the study; the participants were in the 4th to 39th week of pregnancy. Susceptibility to CMV infection was determined by anti-CMV immunoglobulin (Ig) M and IgG immunoassays.

Results: The mean age of the women were 29 ± 6/5 years. On the basis of the results, 326(94%) of women were classed as immune to CMV virus infection; however, the prevalence of IgG anti-cmv virus antibodies measured in the participants' serum was 94%. The prevalence of IgM anti-CMV virus antibodies measured in the participants' serum was 29 out of 348(5/3%).

Conclusion: Although the incidence of CMV is low we suggest the antenatal screening and vaccination of all females of child bearing age to eliminate this potentially devastating virus in the Kashan.

Keywords: CMV, antibodies, pregnant

P474: **Determination of PT toxocity of B.pertussis using CHO-cell assay**

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Background and Aim: Whooping cough is an acute contagious respiratory infection caused by Bordetella pertussis. B. pertussis mainly affects respiratory tract in human and is caused dead in newborn by acute respiratory disease. Vaccination is recommended as the most important way to encounter the disease. Current and available vaccines are prepared from the whole killed bacterium and also acellular containing important purified antigens to control the disease. Safety, quality and efficacy are the most important concerns in vaccine production. Toxicity of a biological product, which shows one of the aspects of safety, is determined by in vivo and in vitro methods. One of the World Health Organization (WHO) more recently concerns and recommendations in vaccine production is the 3Rs; Replacement, Reduction and Refinement. Reducing the animal suffering, costs and variability of results, are some of the reasons to replace or move on from in vivo tests to in vivo. In current study, pertussis toxin toxicity was measured by a cell culture method, as an in vitro, in place of Mouse Weight Gain Test (MWGT), as an in vivo, and the two methods sensitivity were compared.

Methods: In brief, the pertussis vaccine from Razi Inst. was used to measure the quantitative amount of toxin before and after detoxification, if any remained. Bacterial identifications were performed in several steps in vaccine production processes using Gram staining, agglutination tests and bacterial culture on Bordet-Gengue (BG). To perform MWGT, serial dilutions of vaccine samples in PBS and only PBS as negative control were prepared. In parallel, cell culture technique was utilized to compare the quantitative measurement capacity of this method as invariable in vitro with MWGT. The cellular cultures were therefore prepared from Chinese Hamster Ovaries (CHO) cells in flasks and then 3×104 cells were transferred into each 96-well plate. After observing the confluent monolayer cells, the supernatant RPMI medium was discarded then washed with PBS. Next, 50 µl of each serial dilutions of vaccines prepared were added to the wells in triplicates at 37 °C for 24h in the CO2 incubator and lastly cell clustering effect was examined at 6h intervals for 24h. Accordingly, Serial dilutions of anti-PT were mixed up with variable amounts of bacterial counts, as the antigen concentrations, in neutralization assays for 1h incubation at 37 °C in the CO2 incubator to find out the cutoff point (1: 128).

Results: 75µl of anti-PT neutralized 2×104 bacterial antigens. Finally, serial PT dilutions were performed and mixed with 75µl of anti-PT, then added to the confluent monolayer cells to observe and measure the neutralization effect of PT.

Conclusion: 75µl anti-PT neutralized 2×104 bacterial antigens which could neutralize 3.75 nanograms of PT in the neutralization assays. It will also be concluded that cell culture method (CHO cell assay) can be employed to indirectly and quantitatively measure the amount of PT in the process of whole cell and acellular pertussis vaccines production.

Keywords: vaccine,pertussis,toxicity,CHO-cell,MWGT

P475: **Molecular Detection of Aggrigatibacter actinomycetemcomitans and Porphyromans gingivalis in Gingival Infections of Orthodontic Patients**

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Background and Aim: Bacterial plaques are the important etiologic factors of gingival(periodontal) diseases and dental caries. Above mentioned illnesses are common diseases of oral cavity, thus, these diseases are important factors for loss of teeth. Most studies have reported the specific bacteria as infectious agents responsible for these diseases, and indicated that disruption of oral ecological equilibrium by the action of numerous external and internal factors are correlated to development of plaque of the especial pathogens. It is well established that orthodontic appliances(bracket, band and arch wire) create additional retentional surfaces and spaces in oral cavity, playing an important role in growth of these plaques, therefore, periodontal diseases and dental caries are the main problems of orthodontists and these patients. So that, recognition of etiologic factors of these diseases is important for prophylaxis and cure performances, also the most of periodontopathogens were gram negative and anaerobe bacteria, moreover, molecular techniques are more suitable than cultural method for detection of these pathogens. The aim of present study was molecular detection of two major pathogenic bacteria: Aggrigatibacter actinomycetemcomitans and Porphyromans gingivalis in plaques of gingival infections in patients treated with fixed orthodontic appliances.

Methods: By continually referring to the orthodontic department of three dentistry clinics of Karaj city during six months, microbiological sampling was performed from subgingival plaques of 65 patients with clinical manifestation of gingivitis treated with fixed orthodontic appliances. Patients had no systemic diseases, no smoking and no use of antibiotics during 3 month period before the sampling. Whole genomic DNA of the samples was extracted using DNPTM kit(CinnaGene Co, Iran) and a 16S rRNA gene segment- based multiplex PCR method was used to simultaneous detection of Aggrigatibacter actinomycetemcomtans and Porphyromans gingivalis. PCR products were visualized by agarose gel electrophoresis using SYBR Green stain and DNA size Marker)1000 bp Plus DNA Ladder Vivantis,CinnaGen Co,Iran).

Results: Molecular evaluation of this study showed that Aggrigatibacter actinomycetemcomtans was not present in tested samples, although, unusual presence of porphyromans gingivalis with frequency of 10.7±0.3% was reported.

Conclusion: Porphyromans gingivalis is the putative etiologic factor of gingival diseases. Presence of this pathogen in gingivitis lesions of orthodontic patients with movement of teeth and increase of osteoclasts activity during this therapy increases the risk of gingival lesions progress, therefore, we can conclude and recommend orthodontists to pay more attention to this pathogen for improving plaque control, curing gingivitis and preventing the progress of the lesions by appropriate therapeutic protocols.

Keywords: Multiplex PCR, Aggrigatibacter actinomycetemcomitans, Porphyromans gingivalis, gingivitis, orthodontic appliances.

P476: **Comparison between phenotypic and genotypic characterization of antibiotic resistant in E.coli bacteria isolated from human feces**

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Background and Aim: Escherichia coli is used as an index for determinding feacal contamination in water and foods. Also Ecoli is one of the most important pathogen of intestinal and urinary tracks.The aim of this study was to compare the phenotypic and genotipic characteristics of the antibiotic resistance in Ecoli isolated from human

Methods: Sensivity and resistance of 114 isolates of Ecoli were determined with disk diffusion test. Indeed some of the resistance genes were investigated in the isolates using PCR method. The antibiotics were Ampicillin, Tetracycline, Trimethoprim, Gentamicin, Chloramphenicol and Ciprofloxacin.

Results: The results showed that the lowest resistance was for Gentamicin (0%) and the most resistance was for Trimethoprim (79/8%). The antibiotics resistance for Cotrimoxazole, Ciprofloxacin, Ampicillin and Tetracycline 71/05%, 10/5%, 52/63%, 3/5% respectively. The PCR results showed 10 isolates contain sul1 ,49 isolates contain citm, 8 isolates contain tetA, 36 isolates contain dfrA1, 11 isolates contain qnr genes but there was no isolate with aac(3)-IV gene.

Conclusion: comparison between phenotypic and genotypic of the isolates revealed that citm, tetA, dfrA1, qnr, sul1, aac(3)-IV genes covered 42/98%, 7/01%, 31/57%, 9/64%, 8/7%, 0% of the antibiotic resistancy , respectively.

Keywords: Ecoli, diarrhea, antibiotic resistance, antibiotic genes.

P477: **Survey on antibiotic resistance of the E.coli isolated from gastroenteritis cases in Shahrekord.**

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Background and Aim: Escherichia coli is one of the important causes of diarrhea in children, adult and immunecompromised ones in developing country. The aim was to investigate antibiotic resistance of E.coli isolated from gastroenteritis cases in Shahrekord, in order to obtain the rapid and suitable treatment.

Methods: This survey was a cross-sectional study. 234 fecal samples were collected from diarrheic patients in 1390. Bacteriological and biochemical tests were used to identify E.coli. Antibiotic resistance of the isolates was assessed by Disk diffusion method.

Results: The results showed that the minimal resistance was to Gentamycin (0%) and the maximal resistance was for Trimethoprim (79/8%). The resistance for Ciprofloxacin, Ampicillin, Tetracycline, Chloramphenicol, Sulfametoxazole and Cephotaxime were 10.5%, 52.63%, 3.5%, 3.5%, 71.05%, 7.01% respectively. Also the results revealed that the single, double, triple and tetraple antibiotic resistance among the isolates were 17.5%, 39.5%, 38.6% and 4.4% respectively.

Conclusion: According to the results of this study using of Trimethoprim and Sulfametoxazole is not suitable in E.coli gastroenteritis in this area, while Ciprofloxacin and Cephotaxime may use as a suitable antibiotics in these cases.

Keywords: E.coli, Diarrhea, Antibiotic resistance, Disk diffusion, Shahrekord.

P478: **The use of multiplex PCR method in detection of Helicobacter pylori: Comparison with Rapid urease test and Histological examination**

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Background and Aim: Helicobacter pylori infects about half of the world's population. It has been linked to gastritis, peptic ulcers disease, and gastric adenocarcinoma. There are several invasive and noninvasive tests for diagnosis of H. pylori infection. Endoscopy is used to obtain biopsy material for rapid urease test, histo-pathological examinations, and DNA analysis. Methods based PCR are considered highly specific and sensitive tests. In recent studies used a multiplex PCR method to detect different H. pylori genes and their allelic variants that are associated with different stages of H. pylori virulence. In this study, two pairs of primers: HP1 and HP2 specific for Helicobacter genus, cagA1 and cagA2 specific for Helicobacter pylori species were used. The aim of the study was to evaluate the use of multiplex PCR analysis to detect the different H. pylori genes. Any sample positive on histological examination as well as rapid urease test was considered as the gold standard for determination of the sensitivity and specificity of the PCR methods.

Methods: Patients: Gastric biopsy materials were collected from one hundred patients with different gastrointestinal disease who referred to Tabriz Emam Reza hospital. Tissue Specimens: At least three antrum (pyloric gland area) biopsy specimens were taken from each patient for rapid-urease test, histology and an additional antrum biopsy specimen was obtained for PCR analysis. DNA Extraction: DNA extraction was performed using the DNG -Plus kit (CinnaGen Co, IRAN). Primers and PCR Conditions: PCR was carried out with primers specific to the adhesin subunit gene.

Results: Preliminary results are provided and the final results will be presented in Congress. Genomic DNAs were extracted from all strains. 50 samples were examined by multiplex PCR method.

Conclusion: Discussion after obtaining the final results will be presented in Congress.

Keywords: Helicobacter pylori, Multiplex PCR method, Histological examination, Rapid urease test

P479: **Prevalence of hepatitis B and hepatitis C, and their risk factors among patients in Ahwaz medical diagnosis laboratory**

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Background and Aim: Hepatitis B and C virus are the major causative agents of acute and chronic liver disease worldwide and are believed to be responsible for a million deaths annually. These viruses have the same transmission routes, dual infection may occur. Thus, all donated units and blood products are tested for Hepatitis B and C. The aim of this study was to determine the seroprevalence of HCV and HBsAg in patients Ahwaz medical laboratory from 2011 till 2013.

Methods: Serum samples were collected from 420 patients who were applicant for hepatitis B and C viruses. At first, a questionnaire containing some questions about demographic characteristics was filled for all patients, including: age, sex, and frequency of blood transfusion in year, time of diagnosis, history of vaccination, then blood samples of patients were tested to detect serum markers including HBsAg, HCVAb with ELISA method.

Results: 4 (0.95%) patients were HBsAg positive and 7 (1.7%) HCVAb positive. There were 9 (81.8%) males and 2 (18.2) females. The rates of both were higher in males. Seropositivity of HBsAg and HCV was 1 in females and were 3 and 6 in male patients respectively. The mean age was 42.31±12.40 years. The most common risk factors were in order history of surgery and the history of dental procedure in the past decade.

Conclusion: The seroprevalence of co-infection with hepatitis B and hepatitis C virus in our study was lower than worldwide prevalence (>10%). Our results showed the high prevalence of hepatitis B and C in patients as blood recipient. Due to several risk factors in blood donors, more studies are recommended in order to evaluate the correlation between prevalence of infections with one single risk factor as an independent variable without any factor intervening factors. Given the importance of the screening of hepatitis B and C in blood donors, using sensitive diagnostic tests, especially molecular tests, for reduction in the window period is critical. These findings highlight the necessity of public policies to control hepatitis B and C infections.

Keywords: Hepatitis B, Hepatitis c, ELISA, Ahwaz

P480: **Evaluation of Acinetobacter spp. isolates from burned patients for harboring plasmid**

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Background and Aim: Treatment of nosocomial infections is difficult due to increasing level of antibiotic resistance and probably carriage of plasmids by some bacteria could be considered as a special factor for occurrence of high level of antibiotic-resistant character. The present study was conducted to evaluate the antibiotic resistant and occurrence of plasmid in Acinetobacter isolated from wound of burned patients.

Methods: Two hundred twenty constitutive clinical samples were collected from hospitalized burned patients in Isfahan Burn Hospital during three months priod (Mar 2012 to Jun 2012). Identification of Acinetobacter baumanii was performed using standard biochemical test and then confirmed with Api 20E. Antibiotic resistant was determined by standard Agar disk diffusion against three groups of antibiotics viz., meropenem, Ceftriaxon, Ciprofloxacin. Then plasmid profile was detected by column method, respectively. Furthermore, Plasmid curing was done on these strains using ethidium bromide. In the study antibiotics Ciprofloxacin, Ceftriaxone, Meropenem were as resistance markers for plasmid curing.

Results: The results obtained from this study indicated that 30 Acinetobacter baumanii isolates were resistant to three antibiotics tested. The results indicated that the frequency of occurrence of the plasmid in the isolates was 60%. The Strain of Acinetobacter baumanii lost their plasmid due to cure by the ethidium bromide at 125 and 250µg/ml. The cured strain of Acinetobacter baumanii was sensitive to ciprofloxacin (83%), ceftriaxone (60%) and meropenem(33%).

Conclusion: On the other hand, ciprofloxacin, ceftriaxon and meropenem resistant characters probably are plasmid mediated and therefore these genes could transmit among bacteria easily. Overall, antibiotic-resistant character in infectious agents might be related to plasmid and therefore, it could transmit effortless among the bacteria especially in burn hospitals.

Keywords: Acinetobacter Baumanii, Antibiotic Resistance, Plasmid curing, Burned patients

P481: **Microbial pattern and antibiotic susceptibility of agents isolated from nosocomial infections, staff and equipment of Imam Khomeini hospital, Ilam, Iran**

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Background and Aim: Resistant bacteria in various sections of hospital as a main issue of infection transmission are undeniable. Thereby, to achieve infection disease control on hospital, bacteria recognizing and antibiotic resistance determination is necessary. This study is aimed to determine the pattern of microbial resistant of agents that isolated from surgery and ICU wards and comparison of them with nosocomial infection isolates.

Methods: In a descriptive and cross-sectional study, through 6 months samples were isolated from devices and personnel of surgery and ICU ward. Bacteria were diagnosed base on Standard criteria of CLSI protocol. Antibiotic susceptibility were done according to Kirby-Bauer disk diffusion.

Results: 130 samples were isolated, the most frequency of isolates in both wards were Staphylococcus saprophyticus. Most frequent bacteria in ICU and Surgery ward were involved: Entrobacter (35%) and E.coli (25%), in a row. Frequent samples were comprised: Lesion (45%), Sputum (42.5%) and Urine (12.5%). E.coli, Klebsiella and Staphylococcus aureus that isolated from ICU shown high resistance to Amplicillin, Ceftazidime/Cefotaxime, Ceftriaxone, respectively. Whereas bacteria that isolated from Surgery ward shown high resistance to Tetracycline, Amoxicillin- Tetracycline, Ceftriaxone.

Conclusion: Regarding to resistance pattern, there is a correlation between resistance of species that isolated from personnel and devices with nasocomial isolates. Therefore, it could be concluded that devices and personnel have main role to disseminate infection. Thereby, the proper disease control policy could be so useful to combat with issue.

Keywords: Antibiotic susceptibility, Resistant Bacteria, ICU, Surgery, Hospital acquired

P482: **Frequency of the bacterial agents in urinary tract infections and their antibiotic susceptibility patterns in pregnant women reffering to Healthy and Medical Centres in Meshkin Shahr**

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Background and Aim: Urinry tract infections(UTI)S present the most common infection in pregnant women. Pregnant women are at increased risk for U.T.I(starting in week 6 through week 24).The causative organisms that are isolated in UTI, are Enterobacteriaceae encompass most colonizing organisms. Further patogenes include gram-positive organisms. The susceptibility pattern of organisms to antibiotics varies geographically.The aim of this study is to determination the frequency of the bacterial agents and their antibiotic susceptibility pattern

Methods: The samples needed for this study collected from healthy center laboratory of Meshlin Shahr. All specimens performed mid-stream of urine and 0.01 ml of each of Symptomatic patients and 0.001ml of each of Asymptomatic patients cultured through two calibrated loop on Blood agar and EMB agar.Isolated strains were identified by standard biochemical tests. Antibiotic Susceptibility Test was performed by Kirby-Bauer standard method.

Results: 4375 of patients were studied. 529 persons(12.1%) showed positive cultures. The frequency of the bacterial agents in 529 strains were as follws: E.coli 328(62%), Coagulase negative Staphylococci 154(29.1%), Morganella morganii 62(11.6), Klebsiella 15(2.8%), Enterobacter 14(2.65%) and Enterococcus 7(1.32%).The results of antibiotic sensitivity rates against 8 used antibiotics were as follows: ciprofloxacin 98.5%, gentamicin 96.6%, nalidixic acid 56.2%, nitrofurantoin 79%, co-trimoxazole 58%, ampicilin 28%, cephalexin 44% and ceftizoxime 63%.

Conclusion: Times New Roman (Headings CS)

Keywords: Urinary tract infection; Antibiotic susceptibility; E.coli; ; Morganella morganii

P483:: **Identification of salmonella gallinarum & pullorum by PCR-RFLP**

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Background and Aim: Salmonella enterica serotype Gallinarum (SG) and Salmonella enterica serotype Pullorum (SP) are non-motile host-specific avian pathogens. Salmonella Pullorum causes Pullorum disease. Salmonella Gallinarum causes fowl typhoid. Some countries are considered free from SP and SG.however, these are sometimes reported, and are still a matter of concern in the poultry industry. They are very similar, and cannot be distinguished by conventional serological and biochemical methods. The standard methods take approximately 5 to 7 days, and are very time-consuming and expensive. biochemical methods have been complemented by DNA-based molecular techniques, because of their sensitivity, specificity, and swiftness. Such methods include restriction fragment length polymorphism (RFLP), which is sometimes associated to PCR (PCR-RFLP). Most Salmonella strains have two structural genes (fliC and fliB) that encode flagellins. Non-motile strains generally exhibit these structural genes, but are unable to build up a functional flagellum. Aim: The objective of the present study was to differentiate SP and SG isolated in Iran, by PCR-RFLP using Hinp 1I enzyme.

Methods: The bacterial used in this stady were obtained from Razi Vaccine &Serum Resarch Institute of Karaj. 1.Culture of bacteria 2.DNA extraction 3. PCR primers: The following two primers were used for the amplification of flagellin gene phase 1(FliC): 4.PCR according fliC gene 5.Gel electrophoresis(197 bp) 6. PCR-RFLP with Hinp 1I enzyme 7.Gel electrophoresis(197 bp or115 and 82 bp based on bacteria)

Results: Amplification of the fliC gene. The expected 197 bp fragment of the fliC gene was successfully amplified from all Salmonella Gallinarum and Pullorum strains. PCR-RFLP analysis: Digestion of SG amplicons with Hinp 1I yielded two bands, of 115 and 82 bp, while no change in SP amplicons was observed, since no digestion occurred

Conclusion: Salmonella Gallinarum biovar Pullorum and S. Gallinarum biovar Gallinarum are considered important pathogens, causing, respectively, Pullorum disease and fowl typhoid in poultry . S. Gallinarum biovars Gallinarum and Pullorum represent the same serovar but different biovars, their identification and differentiation is based mostly on biochemical characteristics. Therefore, DNA-based methods, especially those based on PCR, were used for the differentiation of S. Gallinarum biovar Gallinarum from S. Gallinarum biovar Pullorum. Of these methods, analysis of the phase 1 flagellin C gene (fliC) by PCR-RFLP seems to be the most promising because of its sensitivity, specificity, and speed. The PCR-RFLP system has been frequently used in differentiation techniques because it is cheap and easy to perform. In our study, the fliC gene in SP and SG were amplified. PCRamplicons(197bp) were digested with the Hinp1I enzyme. Two fragments were obtained (82bp and115bp) for all SG strains, whereas no digestion was observed in the SP strains. In the present study, we were able to demonstrate that the use of fliC gene restriction patterns is an useful method to allow the differentiation between strains of S. Pullorum and S. Gallinarum isolated in Iran, including those with atypical biochemical behavior. Therefore, our results reinforce that this method may be adopted to differentiate SP from SG.

Keywords: FliC gene, differentiation, Salmonella Gallinarum, Salmonella Pullorum

P484: **Inhibitory effects of Citrus aurantium extract in comparison with Cotrimoxazole and Nitrofurantoin on the bacteria isolated of Urinary Tract Infections in 2012**

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Background and Aim: In discriminate use of antibiotic in our society today has led to appears resistance to antibiotics.This survey consider to use the extract and essence of the Citrus aurantium (which have a so many rate of planting in Iran) and also survey on extract on bacteria whose cause Urinary Tract Infections(UTI), and compare this with common antibiotics prescription.

Methods: This research is an experimental study.Citrus aurantium extract and essence performed with boil water by maceration and hydrodistilation by clevenjer methods, respectively.We collected urine from patients and then cultured on blood agar, MacConkey and EMB media at microbiology laboratory of Shahid Beheshti University of Medical Sciences. Methicillin resistance Staphylococcus aureus (MRSA), Streptococcus agalactie , E.coli , Klebsiella pneumonia and Enterococcus faecalis was diagnosed with special test including catalase, oxidase, CAMP, hemolysis, mannitol salt agar test and IMVIC.Two methods had used. First was disc diffusion test and second was Minimal Inhibition Concentration(MIC). The blank discs were macerated in all of extracts and essences then culture on Mueller-Hinton agar with two different antibiotic discs (Cotrimoxazole, Nitrofurantoin), to compare inhibition effects of extract and essence with antibiotics .Minimum Inhibitory Consentration method have been placed in 9 tubes in different dilution(1/2-1/512) and 0.1 cc from 0.5McFarland of bacteria was added in all tubes after 24 hours incubation, each tube was cultured on Mueller-Hinton media. The MIC& NBC (No Bacterial Culture) were detected.

Results: Enterococcus faecalis had 100% sensitivity to extract and cotrimoxazole but 90% to Nitrofurantoin .E.coli had 100% sensitivity to Cotrimoxazole but 90% to Nitrofurantoin and it was totally resistance to extract and essence. Klebsiella pneumonia had 90% sensitivity to Nitrofurantoin, 80% to Cotrimoxazole and resistance to extract and essence.Streptococcus agalactie was 100% sensitivity to essence and cotrimoxazole but 90% to Nitrofurantoin and shown 80% sensitivity to extract. MRSA shown 100% sensitivity to Nitrofurantoin and Cotrimoxazole and 70% sensitivity to essence and extract. Extract and essence had inhibition effect on Entrococcus faecalis and Streptococcus agalactie till 1/128 in tube method.

Conclusion: After all, we totally conclude that essence and extract of Citrus aurantium have a much more effectiveness on gram positive bacteria in comparison with gram negative bacteria.

Keywords: Nitrofurantoin,Cotrimoxazole,Citrus aurantium,Urinary Tract Infection.

P485: **ENSERAF AZ ERAEYE IN MAGHALE ; Prevalence of Staphylococcus aureus isolated of endotracheal tube of patients in toxicological ICU of Loghman Hospital in 2012**

Masoud Dadashi[1], Gita Eslami[2], Hossein Goudarzi[2], Fatemeh Fallah[2], Soudabeh Taheri[2], Ebtehaj Heshmatkhah[3], Zahra Zahirnia[1]

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Background and Aim: FAGHAT MAGHALEYE ACCEPT SHODEYE AVVAL BA ONVANE: Inhibitory effects of Citrus aurantium extract in comparison with Cotrimoxazole and Nitrofurantoin on the bacteria isolated of Urinary Tract Infections in 2012: RA ERAE MIDAHAM.

Methods: FAGHAT MAGHALEYE ACCEPT SHODEYE AVVAL BA ONVANE: Inhibitory effects of Citrus aurantium extract in comparison with Cotrimoxazole and Nitrofurantoin on the bacteria isolated of Urinary Tract Infections in 2012: RA ERAE MIDAHAM.

Results: FAGHAT MAGHALEYE ACCEPT SHODEYE AVVAL BA ONVANE: Inhibitory effects of Citrus aurantium extract in comparison with Cotrimoxazole and Nitrofurantoin on the bacteria isolated of Urinary Tract Infections in 2012: RA ERAE MIDAHAM.

Conclusion: FAGHAT MAGHALEYE ACCEPT SHODEYE AVVAL BA ONVANE: Inhibitory effects of Citrus aurantium extract in comparison with Cotrimoxazole and Nitrofurantoin on the bacteria isolated of Urinary Tract Infections in 2012: RA ERAE MIDAHAM.

Keywords: FAGHAT MAGHALEYE ACCEPT SHODEYE AVVAL BA ONVANE: Inhibitory effects of Citrus aurantium extract in comparison with Cotrimoxazole and Nitrofurantoin ... RA ERAE MIDAHAM

P486: **Determination of predominant genotype of Helicobacter pylori in patients with gastroduodenal in Iran north/ tonekabon city**

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Background and Aim: Helicobacter Pylori colonize in gastric mucosa and causes disorders in upper gastro intestine. The clinical outcome of Helicobacter pylori infection may be associated with or without Cag A or ice A genes.

Methods: To investigate presence of cag A and ice A genes in Helicobacter pylori strains among patients with gastritis, Gastric ulcer and duodenal ulcer, 80 gastric and duodenal biopsy specimens were taken. Urease test was perfomed for all specimens that H.pylori strains were isolated from 25 patients. We Perfomed DNA extraction used allelic specific PCR to examine iceA and Cag A status of H.pylori isolates.

Results:: H. pylori was isolated in 25 cases. the PCR method indicates prevalence of iceA gene in strains isolated from patients with gastritis, gastric ulcer and duodenal ulcer was 25%, 50% and 25% respectively. Additionally iceA1 gene was found in 75% of the isolates strains and it was predominant. Prevalence of cagA genes in strains isolated from patients with gastritis, Gastric ulcer and duodenal ulcer was 36/36%, 50% and 16/16% respectively.

Conclusion: According to the finding it is suggested the prescense of cagA gene in strains of Helicobacter pylori may play an important role in severity disorders or peptic ulcer. But in conclusion we cannot be suggested any of these gene tobe an determining the nature of clinical disease byH. pylori infection, Various geographiced region,may be associated with different frequency in cagA and iceA.

Keywords: Helicobacter pylori , cag A gene, ice A gene, peptic ulcer.

P487: **Comparison of expression of a tripartite chimeric gene in two hosts**

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Background and Aim: Enterotoxigenic Escherichia coli (ETEC) are an important cause of children's diarrhea in and travelers to developing countries. The release of heat-labile (LT) and/or heat stable (ST) enterotoxins following colonization of intestine produces the symptoms associated with the infection. Bacteria adhere to intestinal epithelial cells via fimbrial and/or afimbrial structures called colonization factor (CFAs) or coli surface antigens (CSs) of which more than 25 have so far been identified. It has been shown that these structures are immunogenic and antibodies raised against them are protective.

Methods: In this study a synthetic chimeric gene coding for the minor pilins of CS1 (CooD), CS2 (CotD) and the N-terminal segment of CFA/I (CfaE) was synthesized and cloned in pBAD/gIIIA. The recipient E. coli Top10 was induced and the amount of arabinose needed optimized.

Results: Western blot analysis showed that the protein was expressed, but in low amount which could have been due to the presence of a large number of cysteins in the protein. To overcome this problem an E. coli host with inactivated thioredoxin reductase and glutathione reductase,[Origami(DE3)] which is permissive to the formation of disulfide bonds in the more oxidative cytoplasm was used.

Conclusion: The results obtained showed that an 103 kDa protein with 8 disulfide bonds could be expressed in this host and Origami is more suitable than Top10 for expressing this protein.

Keywords: ETEC , synthetic gene , fusion protein

P488: **Detection of Mycoplasma pneumonia by Loop mediated isothermal amplification (LAMP)**

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Background and Aim: Mycoplasma pneumoniae is the causative agent of atypical pneumonia and is also responsible for other respiratory tract infections such as tracheobronchitis, bronchiolitis, croup and less-severe respiratory-tract infections in older children and young adults. Three methods are available for the routine diagnosis of infections caused by M. pneumoniae: 1) culture, 2) serology and 3) nucleic acid amplification techniques. Recently a novel nucleic acid amplification method reported called loop-mediated isothermal amplification (LAMP), which is capable of amplifying DNA under isothermal conditions with high specificity, efficiency and speed. The purpose of this study is uses from LAMP assay as a fast and accurate method for early diagnosis of M. pneumoniae infections.

Methods: In this study, 100 samples from patients with atypical pneumonia have been collected from hospitals located in Qom, 2012-2013. DNA samples of patients have been extracted using boiling method. Then, specific primers have been designed for LAMP technique by primer explorer ver 4. First, sensitivity tests have been done for LAMP test and then optimized for the samples. At the end, LAMP products have been examined adding SYBR-green.

Results: LAMP test was optimized with the Bst Large fragment DNA polymerase in 66 degree temperature and 60 min. It was specified that suggested sensitivity test is about one particle of Mycoplasma pneumoniae and no result was obtained during specification test by any of tested DNA. The results showed that 73 samples out of 92 were positive for DNA of Mycoplasma pneumoniae in cases of respiratory samples

Conclusion: The LAMP technique is a more available and compatible method. This technique is considered as a useful and rapid system for identifying Mycoplasma pneumoniae infection.

Keywords: Mycoplasma pneumoniae , LAMP , Detection

P489: **Activity of Azithromycin against Acinetobacter baumannii Strains, Including Those Resistant to Imipenem**

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Background and Aim: Acinetobacter baumannii is a nonfermentative gram-negative rod that causes nosocomial infections, especially in intensive care units (4, 5, 11, 13), with increasing frequency. This organism usually affects immunocompromised, ventilator-dependent, or debilitated patients, causing a great number of clinical conditions, including pneumonia, bacteremia, urinary tract infections, wound infections, endocarditis, and meningitis . The mortality of nosocomial infections by A. baumannii is high, reaching 25 to 34% for bacteremia and 40 to 80 for nosocomial pneumonia. Management of A. baumannii infections can be complicated due to the emergence of isolates with multiple-drug resistance including resistance to carbapenems Therefore, it is necessary to evaluate new molecules that are potentially useful against A. baumannii The aim of this study was determined the in vitro activities of Azithromycin and imipenem against 5 isolates of Acinetobacter baumannii, including those resistant to imipenem

Methods: A. baumannii clinical isolates were identified the temperature growth test (44°C). The strains were stored frozen at _80°C in brucella broth containing 20% glycerol until they were tested for susceptibility. five strains from blood agar cultures corresponding to immunocompromised patients were studied. these isolates were characterized molecularly by means of a repetitive extragenic palindromic sequence-based PCR method. For the determination of the MIC a broth microdilution method was used (cation-adjusted Mueller-Hinton broth; Becton Dickinson, Cockeysville, Md.), in accordance with the NCCLS guidelines Imipenem (Merck Sharp & Dohme Madrid, Spain) and Azithromycin(Merck Sharp & Dohme Madrid, Spain)were the drugs tested. MICs were interpreted according to NCCLS criteria for non-Enterobacteriaceae Because there is no approved standard for considering A. baumannii susceptible or resistant to Azithromycin, provisional MIC breakpoints used for this agent were 2, 4, and8 g/ml to designate susceptible, intermediate, and resistant strains

Results: The MIC at which 50% of the isolates were inhibited (MIC50) and the MIC90 for Azithromycin and imipenem were 8 and 16 µg/ml and 32 and 64 16 µg/ml.

Conclusion: These in vitro results show that Azithromycin has good in vitro bacteriostatic activity against A. baumannii, including strains resistant to imipenem

Keywords: Acinetobacter, Azithromycin,MIC

P490: **The prevalence of virulence factors (RmpA and WcaG) among Klebsiella pneumoniae isolates producing CTX-M-1**

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Background and Aim: In recent years, the rapid spread of multidrug resistant Klebsiella pneumoniae has been observed worldwide. The dissemination of resistance is associated with genetic mobile elements, such as plasmids, that may also carry virulence determinants. The aim of this study was to investigate the prevalence of virulence determinants (rmpA and wcaG) among Klebsiella pneumoniae isolated from Tehran hospitals, Iran.

Methods: A total of 114 blaCTX-M-1 group – positive K. pneumoniae strains was included in this study. They were collected between April and December 2011 from different clinical samples in three hospitals of Tehran, Iran and were mostly isolated from tracheal secretions (55.3%), urine (26.3%), and wound (9.65%). Susceptibility of isolates to 14 antibiotic disks was determined by disk diffusion agar method. Polymerase chain reaction (PCR) was used to identify the rmpA and wcaG virulence genes. The transferability of virulence genes was evaluated by conjugation assay.

Results: Most of the isolates showed high level of resistance: cefotaxime and ceftriaxone (100%), amoxicillin-clavulanic acid and aztreonam (98.2%), and ceftazidime (95.6%). Of the 114 blaCTX-M-1 positive isolates, rmpA and wcaG genes were detected in 5 (4.4%) and 34 (30%) isolates, respectively. No isolates featured both virulence genes. The rmpA and wcaG transfer by conjugation was obtained for two and 28 K. pneumoniae isolates, respectively.

Conclusion: The results of this study showed that the prevalence of wcaG virulence gene was high in CTX-M-1-producing isolates. Transferable plasmids carrying virulence genes may also contain antibiotic resistance genes and can be transmitted in hospital environment. Continued monitoring of drug resistance and restriction of antibiotics usage are necessary in clinical settings.

Keywords: Klebsiella pneumoniae, Virulence genes, Drug resistance

P491: **Detection of CTX-M-1beta lactamase genes in Escherichia coli isolated from clinical samples by polymerase chain reaction method in Kermanshah imam reza hospitalple(2012)**

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Background and Aim: Extended-spectrum beta-lactamase of CTX-M-type is considered as an important mechanism resistant to cephalosporin in the gram-negative patogene and is wildly growing. Enterobacteriaceae species are able to produce extended-spectrum beta-lactamases (ESBLs). The purpose of this study was to find the percentage of CTX-M-1carrying E.coli strains in Kermanshah imam reza hospital using Polymerase Chain Reaction method.

Methods: In this sectional-descriptive study, antibacterial susceptibility patterns of 85 Escherichia coli bacteria to Cefotaxim, Ceftazidim, Cefterixon and Azteronam tested using disk diffusion (Kirby-Buer) method. In addition, confirmatory tests for detecting ESBLs phenotypes were performed using Ceftazidim-clavulanic acid and Cefotaxim- clavulanic acid combination disks (MAST). The presence of CTX-M gene was assessed using PCR.

Results: Atotal samples of Ecoli was 85.Confirmatory phenotypic test showed that(48) 56/4% of the strains were ESBL positive. The prevalence of CTX-M gene in isolated Escherichia coli was(29)60/4 %.

Conclusion: In this study High frequency of CTX-M gene in Ecoli strains is a serious matter and would pose a public hazard and every step should be taken to avoid such hazard.

Keywords: Ecoli, Extended-Spectrum β- Lactamases, CTX-M gens.

P492: **A PCR protocol using STM4497 gene as a target for specific detection of Salmonella Typhimurium**

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Background and Aim: Salmonella Typhimurium is a major cause of gastroenteritis and is found in both animals and humans. The oral route through the consumption of contaminated food or drink is the usual cause of Salmonella infection. Since the traditional microbiological method for the detection of Salmonella are time consuming, quicker approaches have been searched. DNA-based methods, such as PCR, have become very popular for microbial detection. The purpose of this study was specific and rapid detection of S.Typhimurium by PCR using STM4497 gene as a target.

Methods: Standard bacterial strain S.Typhimurium (ATCC 14028) used for optimization the test. Bacteria were cultured in Tryptic Soy Broth medium. The genomic DNA was extracted from the broth culture. Following DNA extraction, PCR have been applied for detection of S.Typhimurium, using specific primer pair. STM4497 used as a target gene of PCR amplification.

Results: The result of this study showed that the standard strain produce target fragment. In fact designed primers produced a 357 bp fragment.

Conclusion: In conclusion, PCR is a rapid, sensitive, accurate, and trustful method for detection of S.Typhimurium.

Keywords: Salmonella Typhimurium, polymerase chain reaction, Salmonellosis.

P493: **A PCR protocol using fliC gene as a target for specific detection of Salmonella Typhimurium**

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Background and Aim: The genus of Salmonella is a gram-negative rod shaped bacteria in the family of Entrobacteriacea. Salmonellosis outbreaks involving typhoid fever and human gastroenteritis are important diseases in tropical countries where hygienic conditions are often not maintained. A rapid and sensitive method to detect Salmonella Typhimurium is needed to improve control and surveillance of typhoid fever and Salmonella gastroenteritis. The purpose of this study was rapid and molecular detection of S.Typhimurium by PCR using fliC gene.

Methods: Standard bacterial strain S.Typhimurium (ATCC 14028) used for optimization the test. Bacteria were cultured in Tryptic Soy Broth medium. The genomic DNA was extracted from the broth culture. Subsequently, PCR assay using specific primers was performed for amplification of the target fliC gene.

Results: The result of this study showed that the standard strain produce target fragment. In fact designed primers produce a 743 bp fragment.

Conclusion: Our results showed that the molecular methods like PCR are fast and sensitive for detecting the presence of S.Typhimurium. Furthermore fliC is an accurate candidate gene for laboratory detection of S.Typhimurium.

Keywords: Salmonella Typhimurium, polymerase chain reaction, Salmonellosis.

P494: **Prevalence of nosocomial urinary tract infections in Valiasr hospital in Birjand in 2011-2012**

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Background and Aim: Uti is the most common hospital infection. Prevalence this infectionis variable 15-90% in different studies. Elderly catheterization, dehydration are underlying factors for UTI. Due to important of diagnosis UTI and their treatment this study was done

Methods: Included patients were them hospitalized for more than 3 days and not get antibiotics , no pregnant, and no diabetic. Patients informed from study we get U/A from them and repeat daily .sample get under observation only one nurse.all examed in lab hospital by one microbiologist. data analyzed by X2 and T test.

Results: Prevalence UTI was 21/7%, in internal medicine 10/3%, cardiovascular 39/1%, and in infectious 43/5%. Prevalence in patients were hospitalized less than 5 days was 19/1% and in more than 10 days 41/7%. There was significant differency between pathogenic agent in internal and infectious ward.(P=0/003).

Conclusion: According to the high Prevalence of UTI, we offer U/A was done as routine in different wards.

Keywords: Urinary tract infections, hospital

P495: **Prevalence Oropharynx Colonization in hospitalized patients**

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1- Prevalence Oropharynx Colonization in hospitalized patients

Background and Aim: Nosocomial infections are those infections occur during hospitalization or as a result of hospitalization respiratory. hospitalization respiratory Infections are etiology of 50% mortalities. Colonized micro organisms are the most important factors in this infections. This study was done for evaluation oropharyngial colonization hospitalized patients.

Methods: This descriptive analytical study was done on 100 patients were hospitalized in Valiasr Hospital in Birjand science 2012 The samples taken from pharynx and by transport medium cultured in EMB and Blood Agar by one persons. data collected and analyzed by SPSS.

Results: From 100 patients. 50% internal medicine ward(Heart, internal, infectious) and surjical(general surjery , orthopedy, orology,ENT). This was not significant association between organism and age , sex, diabet. The most common pathogen in ICU was sta, in internal medicine ; streptococcus and sta 40%, and gram negative bacillus 30% but in surjical first gram negative bacillus 65% and second staphylococcal 25%.

Conclusion: In order to control respiratory hospitalization, oropharyngial colonization in hospitalized patients in the most effective rows.

Keywords: hospitalization, colonization, oropharyngial

P496: **Antimicrobial effects of silver nanoparticles**

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Background and Aim: Silver is known for its antimicrobial properties and has been used for years in the medical field for antimicrobial applications. Silver nanoparticles (AgNP) have many important applications including single electron transistors, fuel cells, fluorescent labeling, and DNA/RNA detection via specific probes, as well as potential use in biomedical diagnostic divices, biosensors, nanocomputers, agriculture, and medicine. Recently, because of the increasing incidence of antimicrobial resistance against topically applied “conventional” antibiotic agents, numerous silver-containing products have been developed, mainly for the treatment of wound infections. The antimicrobial activity of Ag nanoparticles was investigated against Salmonella typhi, Pseudomonas aeruginosa, Escherichia coli and Bacillus cereus. In these tests, Muller Hinton agar plates were used and Ag nanoparticles of various concentrations were supplemented in liquid systems. As results, Salmonella typhi and Pseudomonas aeruginosa were inhibited at the low concentration of Ag nanoparticles, whereas the growth-inhibitory effects on Escherichia coli and Bacillus cereus were mild. These results suggest that Ag nanoparticles can be used as effective growth inhibitors in various microorganisms, making them applicable to diverse medical devices and antimicrobial control systems.

Methods: The antimicrobial effect of AgNPs was assessed against a variety of pathogens including Escherichia coli (PTCC 1338), Salmonella typhimurium (wild type), were propagated in LB broth (Merck, Germany) at 37°C and the rest of tested bacteria including Pseudomonas aeruginosa (PTCC1310) and Bacillus cereus (PTCC1015) with agar diffusion technique. The bacterial cells were allowed to grown in 10 ml broth (Muller–Hinton Broth) to give a bacterial concentration of about 4.5×108 CFU/ml, which were incubated at 37°C for 12 h with 200rpm shaking. After incubation centrifug was done at 4000 rpm. For agar diffusion technique, 50 µl of diluted bacterial suspensions was dispensed to the series of agar plates. Wells were cut in the center of the plates and 50 µl of dispersion of AgNP solution at different concentration from 100ppm to 2000ppm was dispensed in each well (6 lg/well). The bacterial cultures and MHB alone and deionized water with NaBH4 were used as controls. The plates were incubated at 37°C for overnight. after 24 h of incubation at 37°C , Zones of inhibition were measured in millimeter and recorded.

Results: The antibacterial activity of silver nanoparticles against Salmonella typhi and Pseudomonas aeruginosa were higher than that against Escherichia coli and Bacillus cereus.

Conclusion: In conclusion, Ag nanoparticles prepared by the cost effective reduction method described here have great promise as antimicrobial agents. Applications of Ag nanoparticles based on these findings may lead to valuable discoveries in various fields such as medical devices and antimicrobial systems.

Keywords: Silver nanoparticle, Antimicrobial, Muller Hinton agar, Deionized water

P497: **Investigation on Frequency of Human Papillomavirus Type 18 infection by PCR among females with cervical cancer in Iran**

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Background and Aim: Cervical cancer remains the second most common cancer in women worldwide and the most frequent in developing countries. However, research has converged to produce unequivocal evidence that cervical infection with certain HPV types is the major cause of almost all cervical cancer cases worldwide. HPV: dsDNA viruses (circular), Widespread in humans and other animal, They cause warts, Some strains 16,18 and 31 associated with high risk of cervical cancer, Transmission of genital tract HPV thru sexual contact. Several cofactors have been described as potential correlates of cervical neoplasia and are now viewed as determinants of progression leading the neoplasic transformation of HPV infections. Among the most important factors are the following: Genetic factors,Immune status,Hormone influences,Tobacco smoking The aim of this study was to Determine HPV18 infection frequency among cervical cancer patient in Iran.

Methods: The method of this study include: 1) sample collection from paraffine embedded cervix tumors lab from pathology department of firoozgar and shohadaye tajrish hospital,Tehran,Iran 2) deparaffination of samples using ethanol and xylenol solutions DNA 3) DNA extraction from tissue samples 4) Quantitation and qualification detection of amplification of Human B-globin gene. 5) Detection of Hpv18 type by PCR using that specific primer

Results: samples of cervical cancer in Iranian patients were collected from2010 to 2013. The age of the patients was from 27 to 75years old. Totally 40 cases were selected for evaluation of HPV18. Among these 40 samples. 1 case was HPV18 positive.

Conclusion: although HPV18 is high risk but it can has low frequency

Keywords: Hpv18, cervical cancer, low frequency, PCR, Human B-globin gene, DNA extraction

P498: **Rapid detection of Haemophilus influenzae by p6 gene polymerase chain reaction**

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Background and Aim: Haemophilus influenzae is an important cause of respiratory infections, including sinusitis, otitis media and chronic bronchitis,which are preceded by asymptomatic colonization of the human pharynx. Haemophilus influenzae is one of the most common causative pathogen in acute bacterial sinusits. The aim of this study is introduces a fast and accurate method for molecular diagnosis of the Haemophilus influenzae sinusitis.

Methods: The specimen was provided from the secretion of maxillary and frontal sinuses of patients from Rasoule Akram hospital. Genomic bacterial DNA was extracted by DNG-plus kit and was amplified employing sequence-specific target namely the p6 gene locus. PCR optimized and carry out sensitivity and specificity tests. Amplicon was cloned and sequenced by Dideoxy chain termination.

Results: The product of optimized PCR with 273 bp length correctly amplified and observed on electrophoreses gel. Evaluation of the selected primers with 8 various DNA demonstrated 100% specificity. Sensitivity of the test was 1 CFU of bacteria. Samples DNA obtained carefully extracted and amplified by PCR. From the 72 samples, 26% (19) of specimens were positive for Haemophilus influenzae.

Conclusion: This study indicates that molecular detection of Haemophilus influenzae employing the p6 gene target is a useful technique in the early detection of Haemophilus influenzae.

Keywords: Haemophilus influenzae, PCR , Sinusitis

P499: **Preparation of conjugated vaccine of Haemophilus influenzae type b polyribosylribitol phosphate with outer membrane vesicle of Neisseria meningitidis serogroup B**

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Background and Aim: Haemophilus influenzae is a gram negative, rod-shaped bacterium that is classified the unencapsulated and the encapsulated. Invasive Haemophilus Influenzae type b (Hib) is an important cause of meningitis and pneumonia in children which possess a polyribosyl ribitol phosphate (PRP) capsule. Like other polysaccharides, the polyribosyl ribitol phosphate (PRP) of the Hib capsule is a T-independent antigen and not immunogenic when administered as a vaccine in infancy. Because the highest rates of disease occur in the first 2 years of life, efficacious Hib vaccines have been designed by covalently linking the PRP capsule to a carrier protein that recruits T-cell help for the polysaccharide immune response and induces anti-PRP antibody production even in the first 6 months of life. In this study, we tried to evaluate a conjugation method by using cyanogen bromide (CNBr) as an activator and adipic acid dihydrazide (ADH) as a spacer . Eventually, in order to increase immunization in vaccine production, we would like to achieve a highly yield from conjugation.

Methods: The first, Hib polysaccharide was activated using cyanogen bromide (CNBr) then were coupled to an adipic acid dihydrazide (ADH) spacer. The activated polysaccharide (AH-PRP) was then reacted with outer membrane vesicle (OMV) of Neisseria meningitidis serogroup B in the presence of EDAC (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide). The conjugated polysaccharides were purified from unconjugated type by gel filtration chromatography.

Results: The conjugate yield was measured as polysaccharide bound to OMV. The result from protein ratio was approximately 33%. We approved that the integrity of the OMV was the same before conjugation by transmission electron microscopic (TEM).

Conclusion: as a result, the findings of this study indicate that this method may is efficient and relies upon the use of adipic dihydrazide as a spacer between the capsular polysaccharide and the carrier protein. It however requires studying more about other methods and comparing them together to achieve an extremely effective conjugated vaccine.

Keywords: Haemophilus Influenzae, vaccine, polysaccharide, immunogenic

P500: **Methylen Blue Based Device for Virus Inactivation in Human Plasma**

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Background and Aim: Despite improvement in safety of plasma transfusion some virus transmission still remains a problem. The windows diagnostic period for some viruses, re-emerged, mutant and new viruses are introduced the most risks to the plasma recipients that, cannot recognized by current diagnostic methods. So as WHO (World Health Organization) recommends, many countries developed PRT (pathogen Reduction Technologies) to inactivate pathogens, especially viruses in plasma components. The MB (Methylen Blue) based methods especially Theraflex® are one of the most famous and universal one. The purpose of this research was designation and manufactures a device that can inactivate viruses in MB environment

Methods: The bag was illuminated by 70 PCs of 1 w (nominal power dissipation) red LEDs from one side. These LEDs emit light at central wavelength of 627 nm with 20 nm FWHM. Two model viruses HSV (Herpes Simplex Virus), VSV (Vesicular Stomatitis Virus) and Polio Virus were used. And TCCID50 were used to calculate virus Log reduction. 1 µM concentration of MB and 4 different illumination times (30, 45, 60 and 75 minutes) were used.

Results: In 1 µM concentration of MB, HSV had 5.20±0.3 maximum log reduction that obtain in 54 J/cm2 after 60 minutes illumination, VSV had 4.90±0.2 maximum log reduction in 70 J/cm2 after 75 minutes and Polio had 1± 0.4 maximum log reduction that obtained in 70 J/cm2 after 75 minutes illumination.

Conclusion: As results show virus inactivation in this method were similar to other methods ($p<0.05$ in comparison to Spring method, and $p>0.05$ in comparison with Theraflex) and it showed this device could inactivate viruses compromise to WHO recommendation.

Keywords: VIRUS INACTIVATION, HUMAN PLASMA, METHYLEN BLUE

P501: **Device Designation for Pathogen Reduction in Human Plasma**

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Background and Aim: Despite improvement in safety of plasma transfusion some virus transmission still remains a problem. The windows diagnostic period for some viruses, re-emerged, mutant and new viruses are introduced the most risks to the plasma recipients that, cannot recognized by current diagnostic methods. So as WHO(World Health Organization) recommends, many countries developed PRT (pathogen Reduction Technologies) to inactivate pathogens, especially viruses in plasma components. The MB (MethylenBlue) based methods especially Theraflex® are one of the most famous and universal one. The purpose of this research was designation and manufactures a device that can inactivate viruses in MB environment.

Methods: 143 pcs of 1W red LED lamp have been used on the device to emit light with wavelength (central wavelength) of 627nm and the width (FWHM full width at half maximum) of 20 nm. Two sides' pouches can be irradiated simultaneously. The distance of the middle of the bag from LED arrays, 4.5cm has considered. This device was designed for light (LED, including the distance and placement of blood bags per LED) with the help of simulation software Wolfram Mathematica® considering the intensity profile (intensity profile) LEDs and right number of used LEDs. Light emission to the plasma bag was optimized and uniformed.

Results: By optimizing the placement of LED, 75% of the radiation was emitted into the bag and the bag limit was less than 40% change in the intensity of the peak intensity. Maximum irradiation power obtained 650 mW / cm2.

Conclusion: In Theraflex® system 180 J/cm2 for 15 min for each bag is used to inactivate pathogens suspended in MB. This device that designed and manufactured can reach required energy level and ready for further inactivation study.

Keywords: PATHOGEN REDUCTION, HUMAN PLASMA, DEVICE

P502: **Correlation between biofilm formation and drug resistance in burn and non-burn clinical isolates of Acinetobacter baumannii**

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Background and Aim: Acinetobacter baumannii has become a major cause of hospital acquired infections mostly due to its antibacterial resistance. Biofilm formation is a well known pathogenic mechanism in A. bumannii since sessile bacteria are protected in an extracellular exopolysaccharide matrix. The purpose of this research was to study biofilm formation in multidrug resistant burn and non-burn clinical isolates of A. baumannii.

Methods: Sixty A. baumannii isolates were employed of which, 30 were burn and 30 were non-burn isolates. Biofilm formation was measured by the microtiter plate assay and susceptibility to 12 antibiotics was measured by disc diffusion.

Results:: Biofilm production was observed in 65% of A. baumannii clinical isolates. Non-burn isolates significantly produced more biofilm compared to the burn strains (59% vs. 41%, $p< 0.05$). All isolates were 100% resistant to cefepime, ceftazidime, cefotaxime, pipercillin, piperacillin-tazobactam, imipenem and gentamicin followed by ciprofloxacin and aztreonam (97.4%), amikacin (93.3 %), mropenem (75%) and tobramycin (33.3%). There was no relation between the potential to form biofilm and multidrug resistance. However, biofilm positive non-burn isolates were significantly more resistant to meropenem and amikacin compared to the burn strains ($p<0.05$).

Conclusion: A higher rate of biofilm production and antibiotic resistance was observed in non-burn isolates of A. baumannii.

Keywords: Acinetobacterbaumannii, biofilm formation, Antibiotic resistance

P503: **Preparation of Staphylococcus aureus capsular type 5 Conjugate to Tetanus toxoid as candidate vaccine with Amidation method in Mouse Model**

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Background and Aim: Staphylococcus aureus is a clinically important capsule-forming bacterium. The capsular polysaccharides are essential for the pathogenesis of and immunity to S. aureus infection and are targets for vaccines. The our aim in this study is conjugate tetanus toxoid protein antigen with S. aureus serotype 5 capsular polysaccharide with Amidation method, vaccine can produce that potential for long-lasting protection and generate memory cells, resulting in immunization of hospitalized patients against staphylococcal Infection.

Methods: The capsule was extracted by Methanol and dialysis method and purified by gel filteration. Rising Glacial acetic acid and autoclaving, capsular polysaccharides was depolymerised. In order to Conjugation CPs with tetanus toxoid using ADH as a spacer and EDAC as linker. The reaction mixture was passed through a sepharose CL-2B column for purification, and did spectrophotometry in 206 and 280 nm, the tubes with maximum absorption were affricate. Non-toxicity of the test antigen, creating a mouse fever, toxicity, sterility was performed.

Results: The study showed that prepared conjugate the ability to react with bacteria antiserum, tetanus anti-toxin and sediment. The creation of the mouse fever test temperature not increase was observed 24 h after injection. So the conjugate is available injection. A sterility test samples are to be sterile after incubation suggest.

Conclusion: The results showed that the extract capsules are active, so it can be used to inject the animal and the induction of antibodies.

Keywords: Amidation, Capsular polysaccharide type 5, Conjugation, Staphylococcus aureus, Tetanus toxoid

P504: **Large-ScaleCulture of Staphylococcus aureus and Its Capsular type 5 Extraction to Produce Antigen for Immunization in Mouse Model**

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Background and Aim: Staphylococcus aureus is seen as a commensal as well as a major human pathogen responsible for a wide range of serious acute and chronic diseases. Bacterial capsules are important virulence determinants for organisms that cause invasive disease. Capsular polysaccharide (CP) has been reported to be present in more than 90% of S. aureus strains. Be our aim in this research isolation of S. aureus capsular type 5 antigen for vaccine.

Methods: S.aureus cultured in the Blood agar for growth. The bacteria inoculated Columbia broth medium for produce capsular polysaccharide. After growing mass of bacteria for extract the CPs first medium centrifuged to long 2 hours at 4°C with rpm 2500 to removed precipitate from the supernatant. The bacteria were suspended in Tris buffer, pH 7.3, and subjected to mechanical agitation with glass beads. The glass beads were removed by filtration. Added 4 times its volume of cooled methanol and kept at 4°C overnight, then centrifuged to precipitate the capsular polysaccharide type 5 containing S. aureus.

Results: The concentrated solution containing the capsule was dialysis 3 times for a day then added about 3 times its volume of cold methanol and centrifuged. The obtained capsule was purified by chromatography with CL-2B gel Sepharose and did spectrophotometry in 206 nm.

Conclusion: The results showed that the purity of chromatographic capsule is approved and used as antigens in vaccine against these microbes is our next target will be reviewed.

Keywords: Capsular polysaccharide type 5, dialysis, extraction, Staphylococcus aureus

P505: **Sensitivity coefficient and death kinetic of streptomycin resistant Brucella abortus in the presence of silver Nano particles and antibiotic-nanoparticle complex**

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Background and Aim: Brucella causes an infectious disease with difficult treatment which contributes to spread of drug resistance and further relapse of infection after treatment .Also there are many side effects to the antibiotics used for treatment. This study aims to design a way to eliminate drug resistance and a way to use a lower dose of antibiotics for the treatment of brucellosis.

Methods: In this study, after the synthesis of silver Nano particles by reduction of silver nitrate, silver Nano-particle antimicrobial properties against pathogenic bacterium Brucella abortus in human was investigated by calculating the sensitivity coefficient and death kinetic of bacterium. Also, the synergistic effect of streptomycin disks (10 micrograms) drenched in the highest concentration of Nano particles without the inhibition effect was investigated during the zero to 360 minute time.

Results: Findings of studying the silver Nano-particle on streptomycin-resistant Brucella abortus showed that only concentrations above 25 mmol inhibited growth of Brucella abortus. Evaluation of the synergistic effect of silver nanoparticles with streptomycin antibiotics showed that plates containing of Streptomycin antibiotic disks with silver Nano particles diluted 12.50 µmol caused the growth inhibition zone according to CLSI. Also the coefficient sensitivity against nanoparticle was 0.006 and was 0.003 against Nano particle-antibiotic complex.

Conclusion: This study showed that silver nanoparticles alone can control the growth of bacteria. It seems that silver nanoparticles with antibiotics, kill drug-resistant bacteria. Using nanoparticles with common antibiotics will enable us to use much lower concentrations of antibiotics and nanoparticles and will lessen their side effects.

Keywords: Brucella abortus, Streptomycin, Silver, nanoparticles, Synergistic, kinetics.

P506: **Ganoderic Acids of Ganoderma lucidum as; Potential Anticancer Agents**

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Background and Aim: Recently, medicinal mushrooms have attracted much attention because of their potent therapeutic activity, especially as antioxidants and immunomodulatory agents. Today's Cancer is the leading cause of death in the world. The current chemical anti-cancer drugs available in market are not target specific and show different side-effects and complications in clinical management of various forms of cancer, which highlights the urgent need for new effective and less-toxic therapeutic approaches. We investigated antioxidant properties of GAs with DPPH and FRAP assays and treated cancer cell lines with GAs which extracted from mycelium of Ganoderma lucidum to show anticancer effects of these metabolites.

Methods: The ganodericacids were extracted from mycelium of ganoderma lucidum with ethanol and chloroform. Different kinds of cancer cells were treated with increasing concentrations of GAs and proliferation was determined. Viability of cells was evaluated by MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) reduction method. Ganodericacids significantly inhibited the growth of cancer cells. Antioxidant properties of GAs were evaluated with DPPH and FRAP assays.

Results: Ganoderic acids clearly demonstrate anticancer activity in experiments with cancer cells and have possible therapeutic potential as a dietary supplement for an alternative therapy for different cancers. Also they show less toxicity for normal cells.

Conclusion: Ganoderma lucidum commonly known as Lingzhi (in Chinese) or Reishi (in Japanese), belonging to family Ganodermataceae has been traditionally used throughout Asia for centuries as a cancer treatment. Ganoderma shows anti-cancer effect alone or in combination with chemotherapy and radiotherapy. A number of bioactive components have been identified from its fruiting bodies, mycelia, spores and culture media. Polysaccharides and triterpenes are two major categories of the bioactive ingredients. Ganodericacids (GA) are a class of closely related triterpenoids (derivatives from lanosterol). There are many of GAs that has been isolated from ganoderma mushrooms, of which GA-A and GA-B are the best characterized. Ganoderic acids possess biological activities including hepatoprotection, anti-tumor effects, and 5-alpha reductase inhibition. The ability of GAs to scavenge the free radicals and to enhance activities of serum antioxidant enzymes indicates its potential use as an antioxidant agent. Ganoderic acids were found to have significant anti-tumor activities. They show these properties by different ways, they can suppress growth (cell proliferation and colony formation) and invasive behavior (adhesion, migration and invasion) of tumor cells.

Keywords: Ganoderma lucidum, Ganoderic acid, antioxidant, anticancer.

P507: **The Influence of Sub- Mic's of Antibiotics on Enzyme Activity and Morphology of Staphyloccus aureus**

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Background and Aim: Subinhibitory concentration (sub-MIC's)of antibiotics although unable to kill bacteria, can effect on enzyme production, its activity and also change the morphology of bacteria to some extent.. The influence of subminimal inhibitory concentrations of penicillin cephalosporin,chloramphenicol and tetracyclin on penecillinase and cephalosporinase production was examined for nine strains of Staphylococcus aureus.Flucloxacillin at a concentration which was capable of stimulation the maximal production of both enzymes was used throughout the experiments.

Methods: One hundred and eighty experiments were performed using concentration from 1/8 to1/32 of the minimum inhibitory concentration of the four antibiotics.The in vitro post sub-Mic effect of cephalosporin and chloramphenicol on E.coli and S.aureus morphology were also evaluated by electron microscope. The kinetics of penicillinse production by S.aureus was studied in the presence of sub-Mic's of penicillin and chloramphenicol at three hourly intervals over 24 hours.

Results: one hundred and eighty experiments, 25 showed an increase Of on enzyme production ,90 a decrease and 65 no effect at any of the above drug concentration .Overwhelmingly stimulation took place at Mic values of 1/8 to 1/32 .On the other hand depression took place mainly at Mic values of 1/2 to 1/4. Four strains whose enzyme activity was unaffected by any of the chosen antibiotics the response was very different.With cephalosporin as a substrate there was no increase in cephalosporinase production for 6 strains.The in vitro study on morphology showed elongation of E.coli and cell wall thickness of S.aureus.

Conclusion: This study showed that penecillinase activity was at the peak after 18 This hours. The number of colony forming units (cfu) was also greatest at this time. Comparison between penicillin and chloramphenicol at 1/8,1/16,and1/32 showed a much greater effect on penicillinase production that did penicillin at similar drug concentrations.

Keywords: Subinhibitory concentration antibiotics, S.aureus, Morphology, Enzyme Production

P508: **The correlation between rifampin resistance and mutations in RRDR region of Mycobacterium tuberculosis clinical strains**

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Background and Aim: Tuberculosis is the most deadly infectious diseases in worldwide. initiation of appropriate therapy is effective in early detection of drug resistance. The purpose of this study was to determine the relationship of resistance to rifampin resistance and mutations has three main codon with Sequences method.

Methods: In this study, of 31 clinical isolates Mycobacterium tuberculosis 23strains resistant to rifampin and 8 of drug-sensitive strains were used. Primers with the help of software, IDT, BLAST, MEGA designed. This primer amplification region of the rpoB gene is associated with resistance to rifampin. Sequences of these fragments were redirected using Applied Biosystem apparatus.

Results: All strains, the band offered 360bp. Sequences results for the detection of possible mutations in codon 516, 526 and 531 rpoB gene The strains showed from 23 strains Rifampin-resistant Mycobacterium tuberculosis 9 strains of mutant strains as one of three codons 516 and 526 and 531 were diagnosed . Mutations in 30% of cases were codon 516 and526 and 60% of cases of codon531.

Conclusion: Determination of codon mutation at 516, 526 and 531 in rpoB gene)RRDR(associated with resistance are Rapid detection of rifampin resistance in Mycobacterium tuberculosis strains in vivo.

Keywords: Mycobacterium tuberculosis,rpoB, rifampin,Sequences

P509: **Histopathological assessment of topical effect of Peppermint oil on infected skin wound with Candida albicans on rats**

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Background and Aim: Candida is a genus of yeasts. Many species are harmless commensals or endosymbiosis of hosts including humans, but other species, or harmless species in the wrong location, can cause disease. Candida albicans can cause infections (candidiasis or thrush) in humans and other animals, especially in immunocompromised patients. In winemaking, some species of Candida can create potential faults in wines. wound infections caused by Candida albicans has grown substantially in recent years. Candidiasis, caused by Candida species, is the most common fungal infection in humans. Beside invasive diseases including candidemia and candidiasis in deep-seated organ, mucocutaneous disorders such as skin and oral candidiasis, vaginal and vulvovaginal candidiasis, have become a problem of significance in clinical practice. Unavailability and expensive drugs, side effects, and particularly, development of drug resistance, led to the use of biological materials to be considered as an alternative solution. Higher and aromatics plants have traditionally been used in folk medicine as well as to extend the shelf life of foods, showing inhibition against bacteria, fungi and yeasts. Mentha species are used for their flavoring and medicinal properties widely throughout the world. Primary investigation on this plant has led to the isolation of polyphenolic acids, several flavonoids and mono terpenoids. Mentha piperita L. (peppermint) is a medicinally important plant that belongs to the family Labiate.

Methods: In this study on 45 male Wistar-albino rats (weight 200±10 g), after general anesthesia, and an wound square with dimensions 1/5 in the 1/5 cm area between the shoulder, immediately was applied to the wound 0.1 ml of the suspension containing 1/5×107 CFU Candida albicans yeast. Then tested in three groups of 15 rats each (control, topical ointment containing 1.5g and 3g Peppermint oil) were randomly distributed into 5 subgroups of 3 rats each (sample groups on different days) groups. During the project was obtained, the end of days 4th, 8th, 12th, 16th and 20th from wounds of different groups, in order to histopathology and yeast counts by a special punch biopsy specimen.

Results: In the skin excisional wound animal model, Peppermint oil at clinical relevant neither dose (1.5 and 3%) promoted infection wound healing. In the yeast counts evaluation, significant reduces the Candida albicans cloning in treatment groups compared control group ($P < 0.0001$). In the histopathology evaluation, neither treatment groups compared control group, could signi?cantly reduce the number of poly morph nuclear cells (PMN as an inflammatory cell), increase the mono nuclear cells and new vessel formation at day four, and also increase fibroblast cells (MNC), at day fourteen after wound creation ($p <0.01$); Although, according to the quantitative factors studied, dose 1.5%, has stronger effects than dose 3%.

Conclusion: Both dose of Peppermint oil plays a preeminent role in the anti-inflammatory, anti fungi and fibroblast-proliferating activities compared control group. Be considered this herbal formula, accelerate infection wound healing and better choice to use a topical ointment has a dose 1.5%.

Keywords: Peppermint oil, ointment, candida albicans, infected wound healing, rats.

P510: **Effect of Quercus castaneifolia on chemotactic behavior of Escherichia coli isolated from gasteroenteritis patients**

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Background and Aim: Chemotaxis is a special behavior of motile bacteria that determine the direction of bacterial movement. In addition, this phenomenon could be considered as an important virulence factor for these bacteria. On the other hand, Acron (Quercus castaneifolia) of the oak tree is a special fruit with therapeutic property. Iranian people usually use this fruit for treatment of the Patient suffering from enteric disease. The present study was undertaken to evaluate the effect of water and hydro-alcoholic (70%) Quercus castaneifolia on chemotactic behavior of 5 strains of E.coli isolated from the gasteroenteritis patients.

Methods: To perform this study minimal inhibitory concentrations (MICs) and Subminimal concentrations (SICs) of the fruit extracts were determined. Then the effect of Quercus castaneifolia extracts on chemotactic behavior of E.coli strains was evaluated by the capillary method.

Results: The results obtained indicated that Quercus castaneifolia extracts inhibit chemotactic behavior of these bacteria. However, the effect of hydro-alcoholic (70%) Quercus castaneifolia extract was more.

Conclusion: In general, chemotactic behavior could be considered a special target for eliminating gasteroeneritis caused by E.coli. Forthermore, Quercus castaneifolia extracts might be use as a remedy for treatment of the patients suffering from enteric disease.

Keywords: Quercus castaneifolia -Chemotaxis -Escherichia coli-gasteroenteritis patients -oak tree-

P511: **Evaluation of metallo-β-lactamases genes in Acinetobacter baumannii isolated from Tehran hospitals with phenotypic and genotypic method**

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Background and Aim: Acinetobacter spp are apportunistic pathogens and are important agents of hospital acquired infections. They have the ability to transmitte genes among themselves and onto other bacteria. This intrinsic capability of these bacteria is important in dissimination of antibiotic resistance genes. The goal of this study was to determine the extent of antibiotic resistance, Minimal Inhibitory Concentration (MIC) value determination, phenotypic determination of metallo β-lactamase production; and finally, genetic identification of the blaIMP and blaVIM metallo β-lactamase genes by PCR method. This study was conducted on Acinetobacter baumannii isolates recovered from clinical cases in various Tehran hospitals

Methods: A total of 176 clinical cases of Acinetobacter baumannii were isolated from various Tehran hospitals in the year 1390. After bacteriological confirmation, the strains were subjected to disk diffusion method in order to determine their antibiotic susceptibility patterns. A total of 10 antibiotic disks were used for this test. Subsequently, Minimal Inhibitory Concentration (MIC) values against imipenem were determined. The imipenem resistant isolates were subjected to Double Disk Synergy Test (DDST) method for phenotypic determination of metallo β-lactamase production. Following the Double Disk method, DNA extractions were performed on the resistant isolates and PCR assays were done using two metallo β-lactamase specific primers (bla-IMP and bla-VIM genes).

Results: The disk diffusion method, which was analysed according to the CLSI guidlines, indicated the percent resistance of the total Acinetobacter isolates to be: Imipenem (97/15%), Ceftazidime (97/15%), Piperacillin-Tazobactam (96/59%), Ticarcillin-Clavolonat (85/22%), Ciprofloxacin (96/02%), Levofloxacin (93/75%), Trimethoprim-Sulfamethoxazol (98/29%), Ceftazidime-Clavolonat (88/06%), Cefotaxime-Clavolonat (96/59%) and Cefotaxime (97/72%). The MIC test indicated that 72/72% of the isolates had MIC values of ?16. A total of 174 imipenem resistant strains were shown to be phenotypic producers of metallo ?-lactamase in the DDST method. The PCR assays indicated that 71 of these isolates carried the IMP gene; whereas, 65 of them harbored the VIM gene.

Conclusion: Our results indicated that the extent of antibiotic resistance is very high among the clinical isolates of A.baumannii in Tehran hospitals. A high percentage of these strains were shown to harbore metallo β-lactamase genes and phenotypically express this resistance trait as well. This is potentially very alarming and necessitate careful administration of antimicrobial agents in clinical setting.

Keywords: Acinetobacter baumannii, Hospital Acquired Infections, Antibiotic resistance.

P512: **Design and application of an improved PCR assay for specific detection of Neisseria meningitides**

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Background and Aim: Neisseria meningitides, a non-motile, aerobic, Gram-negative bacterium and a member of the Neisseriacea family is a causative agent of meningitis. Infection usually begins with nonspecific symptoms including fatigue, fever and headaches but rapidly advances to coma and in about 10% of cases, death. As soon as the meningococcal infection is suspected, the proper antibiotic prescription can be initiated. Culture, Gram stain and other conventional methods to detect the organism are time consuming and unreliable. The aim of this study was to design an improved PCR assay for the specific detection of Neisseria meningitidis.

Methods: The diagnostic specific primers were designed using Allele ID 7.6 software based on the crgA (contact-regulated gene A). The PCR experiment was performed on genomic DNA of N meningitides (ATCC 13060). The product was cloned in the pTZ57R/T vector to construct a plasmid positive control. Restriction enzyme and cycle sequencing analyses were used to confirm the authenticity of the target band. The test specificity was evaluated by performing the assay on the related and non-related bacterial genomes. The sensitivity of assay was tested by making a 10 fold serial dilutions of the plasmid positive control with starting concentration of 70ng/µl.

Results: The PCR assay amplified a DNA fragment with expected size of 500bp. Kpn I - Hind III double digestion and cycle sequencing analysis showed expected bands and sequence respectively. The specificity results showed no cross reaction with the genomic DNA of the other bacteria. 70pg was the lowest concentration of the plasmid positive control that was amplified during the sensitivity testing.

Conclusion: Given the high specificity, sensitivity and rapidity of the assay, it could be further evaluated using the pure culture and clinical samples to apply as a valid in vitro diagnostic (IVD) test.

Keywords: Neisseria meningitidis, PCR, assay

P513: **Isolation and identification of gram-positive bacteria causing ventilator-associated pneumonia in ICU patients shahid Rajai hospital in Tonekabon**

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Background and Aim: Ventilator-associated pneumonia is the most common nosocomial infection in intensive care that increases treatment costs and mortality. The purpose of this study is isolation and identification of gram-positive bacteria causing ventilator-associated pneumonia in ICU patients Shahid Rajai hospital in Tonekabon.

Methods: The study lasted for 6 months, sample of 35 patients hospitalized in the ICU of the Shahid Rajai hospital of Tonekabon that was performed the use of mechanical ventilation for 48 hours. For this purpose, after the departure Nedator of patient lung, by the sterile scissors cut 1 cm from the tube and transferred into the Brain Heart Infusion broth medium and were incubated for 48 h at 37 ° C. After incubation time, grown colonies transferred into the Brain Heart Infusion Blood Agar medium and bacteria were purified by successive cultures and with the advent of grown colonies, the colonies were gram stained and DNA extracted was administered using by phenol - chloroform techniques. Order to DNA amplification was performed PCR test with 16SrRNA universal primers. PCR products were sequenced to identify the isolated bacteria.

Results: From 35 patients under study were isolated 53 bacteria that after performing gram stain were 28 bacteria gram-positive. Identification of gram-positive bacteria isolated by PCR techniques and sequenced demonstrated of presence, 11strains (20/7%) Staphylococcus aureus, 4 strains (7/5%) Corynebacterium, 3 strains (5/6%) Enterococcus faecalis , 2 strains (3/7%), Staphylococcus Haemolyticus, 2 strains (3/7%), Staphylococcus hominis, 2 strains (3/7%), Enterococcus faecium, 2 strains (3/7%), Staphylococcus lugdunensis , 1 strains (1/8%), Staphylococcus epidermidis, 1 strains (1/8%), staphylococcus intermedius in patients hospitalization in the ICU that were used of mechanical ventilation. In 7 patients with ventilator-associated pneumonia from gram-positive was isolated bacteria 2 strains (3/7%) Corynebacterium, 2 strains (3/7%), Enterococcus faecalis, 1 strains (1/8%), Staphylococcus epidermidis. In this study, the prevalence of Staphylococcus aureus in patients with nosocomial pneumonia that require mechanical ventilation, was more than other gram-positive microorganisms that represent a greater tendency is this microorganism to cause pneumonia in patients that Use of mechanical ventilation.

Conclusion:: Gram-positive bacteria isolated from patients multiple cultures with ventilator-associated pneumonia, indicates is a increasing invasion desire and compatibility feature of these bacteria in ICU and easily transition this agents through the devices and personnel to the admitted patients in this section. Identify and assess this gram-positive bacterium abundance causing ventilator-associated pneumonia is necessity in order to therapeutic of determining the policies in the initial collisions and control of infection created by this agents.

Keywords: ventilator-associated pneumonia, Gram-positive bacteria, intensive care unit

P514: **survey the effects of antimicrobial bacteriocin produced by lactobacillus plantarum isolated from traditional cheeses Khorramabad city.**

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Background and Aim: Lactobacillus plantarum, including lactic acid bacteria in dairy products that are heterofermentative. This bacterial can bacteriocin production against pathogen bacteria, as they cause the loss of pathogenic bacteria in the body. The purpose of this review ,survey the effects of antimicrobial bacteriocin produced by lactobacillus plantarum isolated from traditional cheeses Khorramabad city.

Methods: A total of 13 samples were collected from traditional cheeses Khorramabad city in the desired bacteria using phenotypic methods (cell morphology, physiological and biochemical tests) identified and bacteriocin were extracted. Bacteriocin extracted using the method of diffusion, on the pathogenic bacteria Pseudomonas aeruginosa, Proteus vulgaris, Staphylococcus aureus, E. coli, Bacillus cereus and Bacillus subtilis, Streptococcus feacalis was tested

Results: The results showed that the inhibition zone in the bacteria Pseudomonas aeruginosa (12mm), Staphylococcus aureus (10mm), E.coli(11mm), Proteus vulgaris (9mm), Bacillus cereus (7mm), Bacillus subtilis (11mm) Streptococcus feacalis (14mm).

Conclusion: This study showed that Lactobacillus plantarum has an inhibitory effect on pathogen bacterial and improve infection or prevent infection in the body.

Keywords: Lactobacillus plantarum, antimicrobial effects, inhibition zone, bacteriocin

P515: **Antibiotic resistance and antimicrobial effects enterococcus faecium and lactococcus lactis isolated from traditional cheeses Khorramabad city**

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Background and Aim: Intestinal microflora is a complex ecosystem. Introducing new organisms into this highly competitive environment is difficult. Thus organisms that can produce a product or products that will inhibit the growth or kill existing organisms in the intestinal milieu have a distinct advantage. Lactic acid bacteria can bacteriocin production against pathogen bacteria, as they cause the loss of pathogenic bacteria in the body. The purpose of this review there are enterococcus faecium and lactococcus lactis in dairy products cheese and effects of antibacterial and antibiotic resistance this bacteria.

Methods: A total of 13 samples were collected from traditional cheeses Khorramabad city in the desired bacteria using phenotypic methods (cell morphology, Gram staining, physiological and biochemical tests) identified and bacteriocin were extracted. Extracted bacteriocin using the agar well, on the pathogenic bacteria Pseudomonas aeruginosa, Proteus vulgaris, Staphylococcus aureus, E. coli, Bacillus cereus and Bacillus subtilis were examined. In oder hand antibiotic resistance of this bacteria using Antibiogram method were tested.

Results: The results showed that the bacteria Pseudomonas aeruginosa, Staphylococcus aureus, and Ecoli against of bacteriocin production have interrmediate sensitive, the bacteria Proteus vulgaris was sensetive, Bacillus cereus and Bacillus subtilis were resistant. The other hand, the enterococcus faecium against antibiotics kanamycin (30ug) Trimethoprin (250 ug)are resistant and against clindamycin (2ug) tetracycline (30ug)are intermediat sensitive ,against amoxicillin (10ug) and erythromycin (15ug) are sensitive. lactococcus lactis against Trimethoprin (250ug), amoxicillin (10ug), tetracycline (30ug) erythromycin (15ug) are sensetive and against kanamycin (30ug) and clindamycin (2ug) are resistant.

Conclusion: Both bacteria enterococcus faecium and lactococcus lactis have an inhibitory effect on pathogen bacterial and improve infection or prevent infection in the body, these bacteria also have the appropriate antibiotic resistance against most antibiotics that due to having this benefit effects in dairy produce can having consumption and increases safety against pathogenic bacteria.

Keywords: enterococcus faecium, lactococcus lactis, antimicrobial effects, antibiotic resistance, bacteriocin

P516: **Survey Effect of hydroalcoholic extract of the plant yarrow (Achillea millefolium) on some pathogenic bacteria**

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Background and Aim: The used of medicinal herbs among the general population gives rise to the possibility of therapeutic or toxic effect in patients that use these plants. Yarrow is a plant of the genus Achillea., Which has antiseptic and analgesic effects. yarrow (Achillea millefolium), extracted supplied in polypropylene glycol, is reported to function as a biological additive in cosmetic products. This study examines the issue is local Yarrow Extract Green Mountain province has what effect on pathogenic bacteria.

Methods: Plants with characteristics Yarrow (Achillea millefolium) , volume of 30 g was collected and immediately dried in the shade and its hydroalcoholic extract was prepared by the hydroalcoholic method. The extracts be dried, mixed with water to about 0. 5 g / ml and Sterilized with membrane filter and paper discs were treated in a extracted solution Then indicator strains were swapped with sterile culture in Muller Hinton Agar. Then paper discs were dipped into the medium and were placed in the plates with gaps between them and the walls. and then inhibition zone was measured after incubation incubated for 24 h at 37 ° C. then MIC method used later with extracted transferred to Mueller Hinton broth were characterized . all tested as duplicate in the presence of the antibiotic chloramphenicol and amoxicillin were performed as controls. Antibacterial effects on pathogenic bacteria Bacillus cereus, Listeria monocytogenes, Sterptococcus feacalis, Staphylococcus aureus, Pseudomonas aeruginosa, E. coli , Bacillus subtilis and Proteus vulgaris were investigated.

Results: The minimal inhibitory concentration (MIC) for the bacteria Bacillus cereus, Bacillus subtilis, Listeria monocytogenes, Sterptococcus feacalis 250 micrograms per ml for Staphylococcus aureus and E. coli and 125 micrograms per ml for Pseudomonas aeruginosa 75 micrograms per ml of the bacteria Proteus vulgaris 37/5 mg per ml. Inhibition zone of the bacteria Bacillus cereus, Bacillus subtilis, Listeria monocytogenes, Sterptococcus feacalis 8 mm for Staphylococcus aureus, E. coli was 9 mm , for Pseudomonas aeruginosa was 10 mm and for Proteus vulgaris was 13 mm.

Conclusion: The results showed that the extract of Achillea local province had relatively antimicrobial effect to the use as topical particularly is suitable for skin infections.

Keywords: antimicrobial effect, extract, Achillea millefolium, pathogenic bacteria

P517: **Distribution of OXA Carbapenemase Genes in Clinical and Environmental Isolates of Acinetobacter baumannii**

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Background and Aim: Acinetobacter baumannii recently has emerged as a global threat to public health and a major cause of nosocomial infections with high mortality particularly in Intensive Care Units. Carbapenems are important drugs for treating infections caused by multidrug resistant Acinetobacter baumannii. Production of OXA carbapenemases is the major mechanism for carbapenem resistance. The study was conducted to determine the prevalence of carbapenemases and antimicrobial susceptibility among A.baumannii isolates recovered from environment and hospitalized patients of hospitals in Tehran, Iran.

Methods: A total of 222 isolates of A.baumannii were collected from three hospitals during June to December 2010 in Tehran, Iran. Of the 180 specimens collected from hospital environment, 22 were positive for A.baumannii. All of isolates were identified as A.baumannii using conventional microbiological and confirmed by amplification of blaOXA-51-like gene using polymerase chain reaction (PCR). Antimicrobial susceptibility testing carried out by the disk diffusion method. DNA extraction was carried out by phenol-chloroform method. Multiplex PCR was used to detect blaOXA-23-like, blaOXA-58-like and blaOXA-24-like genes. The purified PCR products of OXA-type genes were sequenced using an automated sequencer.

Results: Our results showed that the most of clinical and environmental isolates were resistant to third-generation cephalosporins (ceftriaxone and ceftazidime), fluoroquinolones (ciprofloxacin and levofloxacin), piperacillin/tazobactam and meropenem. The rate of resistance to imipenem was 92% in clinical against 54% in environmental isolates, but all of isolates were susceptible to colistin. Analysis of PCR amplification of blaOXA-51-like gene revealed that, except one, 221/222 (99.55%) clinical isolates had this gene and all of environmental isolates 22/22 (100%) did too. According to Multiplex-PCR results, 122 (55.2%) clinical and 6 (27.3%) environmental isolates carried the blaOXA-23-like gene. Among clinical isolates, 28(12.7%) harbored the blaOXA-58-like and 3(1.35%) the blaOXA-24-like genes. Genes encoding OXA-58, 24 in environmental isolates were not detected.

Conclusion: This study shows a high distribution of blaOXA-23 -like gene in clinical and environmental isolates of A.baumannii. In conclusion, carbapenem resistance may be associated with the presence of blaOXA-23-likegene. This study also indicate high resistance rate of clinical and environmental isolates to antimicrobial agents.

Keywords: Acinetobacter baumannii, OXA, carbapenem resistance, environment, Antimicrobial susceptibility

P518: **Determining the correlation between Presence of integron Class 1 and antibiotic resistance in Enterococcus isolates.**

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Background and Aim: Enterococci, are gr + bacteria, pairs or short chains cocci that can be seen in the stool. These bacteria categorized in the group D streptococci and they are the most common cause of nosocomial infections in the ICU, particularly. A major problem facing enterococci is the resistance to the variety of antibiotics. Enterococci are naturally resistant to antibiotics likes betalactam group, Aminoglycosides, β-lactams and clindamycin. Studies also have shown that drug-resistant enterococci can be encoded by mobile genetic elements such integrons, the transposons and IS and transferred by them. The aim of this study was to Determining the correlation between Presence of integron Class 1 and antibiotic resistance in Enterococcus isolates.

Methods: 150 different clinical samples in 1390 and 1391 were collected from baqiatallah hospital patients, after diagnosis testing and biochemical methods Enterococcus species were determined and isolated. The pattern of drug resistance by the disk diffusion method for determining susceptibility of isolates to Tetracycline, Gentamicin, Erythromycin, Chloramphenicol, Ciprofloxacin, Vancomycin, Linezolide and Tiecoplanin antibiotics were detected. After DNA extraction with using specific primers of class 1 integron was evaluated with PCR method and the pattern of gene cassettes in strain that containing integron were determined.

Results: After the differential arabinose sugar test for identification of two species of Enterococcus faecalis and Enterococcus faecium, in 150 clinical specimens, 58% of cases wrere E.faecalis and 42% of E.faecium were reported. Resistant to common antibiotics in antibiogram test were detected as; 86.58% to Tetracycline, 41.6% to Gentamicin, 65.6% to Erythromycin, 21.8% to Chloramphenicol, 42.7% to Ciprofloxacin, 8.3% to Vancomycin,1% to Linezolide and 2% to Tiecoplanin. Also, the presence of multiple drug resistance in Enterococcus class 1 integron and was considered statistically significant. The PCR reaction for amplification class 1 integron was performed and the results showed that 44% of isolates contain class 1 integron.

Conclusion: The results of this study showed that certain genetic elements especially class 1 integron plays an important role in the transfer of antibiotic resistance between Enterococcus strains and could possibly also transfer this resistance to other bacterial species.

Keywords: Enterococcus faecalis, Enterococcus faecium, Integron ,Gene Cassette

P519: **Antibacterial activity of essential oil of Nepeta glomerulosa Boiss. on some pathogenic bacteria**

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Background and Aim: During last decades, using of natural antibacterial components such as plant essential oils has attracted the attention of many scientific and pharmacological populations. Aromatic plants such as some species of Nepeta genus have traditionally been used in folk medicine, and provide a multitude of flavours and fragrances which have found their way into everyday life. It has been represented in Iran by sixty seven species including thirty nine endemics. Nepeta glomerulosa Boiss. is one of them and used as herbal tea, it has been known as Poone saye Anbooh in Iran. This research studied antibacterial activity of the essential oil of Nepeta glomerulosa Boiss. on some of the pathogenic bacteria.

Methods: The composition of the essential oil hydrodistilled from the flowers of Nepeta glomerulosa collected from Binaloud mountainous in Neyshabur, Khorasan Razavi province, Iran, in June 2011, and was analyzed both by gas chromatography (GC-FID) and gas chromatography-mass spectrometry (GC-MS). Then for evaluation of antibacterial effects of the essential oil, disc diffusion method through the measurement of the inhibitory zone diameter and micro-broth dilution for determining the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) on several standard bacteria, two gram negative bacteria, (Shigella dysenteriae and Salmonella typhi), and two gram positive bacteria, (Bacillus subtilis and Staphylococcus aureus), were used.

Results: Essential oil inhibited growth of all studied bacteria. Among them, zone of growth inhibition was larger in Shigella dysenteriae, which is in consistent with the results of micro-broth dilution method. Minimum inhibitory concentration (MIC) was 4µg/ml for Shigella dysenteriae and Salmonella typhi, and 16µg/ml for Bacillus subtilis, which is equal to MBC. In Staphylococcus aureus, MIC of this essential oil was 8µg/ml and MBC was equal to 16µg/ml.

Conclusion: This research results showed that Nepeta glomerulosa essential oil has a moderate effect on the studied bacteria. Its effect on gram negative bacteria was more than gram positive bacteria.

Keywords: Nepeta glomerulosa, essential oil, Antibacterial activity, disc diffusion, micro-broth dilution

P520: **Does the PRP Polysaccharide conjugate by Tetanus toxoid of Haemophilus influenzae type B stimulates the Humoral Immunity in Rabbits?**

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Background and Aim:: Haemophilus influenza type B (Hib) is the main cause of Meningitis in 4-18 month infants. There are 3 Million new cases each year and its Morbidity & Mortality rate is 400000. The role of Polyribosyl Ribitol Phosphate (PRP) Polysaccharide is significant in Hib immunity. Although, the immune response to PRP is T-Cell independent, such as in other polysaccharides. Since, polysaccharide vaccines show a low efficiency rate, therefore, our aim in this study was to improve the humoral immunity by conjugating PRP to Tetanus toxoid.

Methods:: Haemophilus influenza type B PTCC1623 was cultivated in 10L of CY broth medium in fermentor, and purified by alcohol precipitation method, and by the addition of Cetavlon, and Hydroxyl Apatite. Tetanus toxoid was provided by Razi Vaccine & Serum Institute. 5mg of PRP was conjugated to 10mg of tetanus toxoid by Szu method (2013). In this method, Adipic Acid Di Hydrazide (ADH) was used as the spacer, and 1-Ethyl-3,3-di Methyl Amino Propyl (Carbo Di Amid) (EDAC) was used as the linker. The conjugate obtained was purified by flowing through Chromatography Column, using 4B-CL gel. Immunization was done, choosing 2 groups of white New Zealand rabbits. 25µg of pure PRP in 5ml serum physiology and 25µg of the conjugate were injected to groups 1 and 2, with a 15-day interval, intramuscularly, respectively. The blood was drawn at days 0, 15, 30, 45. The sera were separated, in order to perform Serum Bactericidal Assay.

Results: the results obtained in this study have shown the bactericidal titer of pure PRP was 16. There was no antibody increase in the second injection. The antibody titer of the conjugate was arisen up to 32, by the first injection, and 64 in the second injection. The conjugation rate was 15%.

Conclusion: according to the results, the PRP-TT conjugate and the pure PRP were both able to stimulate the bactericidal antibody. Although, there was no antibody against pure PRP, in the second injection. While, there was a significant antibody titer against the second injection of the conjugate.

Keywords:: Polyribosyl Ribitol Phosphate, Tetanus toxoid, Conjugate vaccine, Haemophilus influenza type B

P521: **Rapid Determination of gyrA and parC Mutations in fluoroquinolone-resistant clinical isolates of Acinetobacter baumannii by the ARMS-PCR analysis.**

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Background and Aim: Acinetobacter baumannii has now become a major cause of hospital-acquired infections worldwide. Most A.baumannii infections occur within intensive care units in patients with serious underlying disease. Among A.baumannii isolates, resistance to fluoroquinolone (e.g., ciprofloxacin and levo-floxacin), is escalating. Mechanism of resistance to fluoroquinolones in Acinetobacter, is mutations in the genes gyrA(S83L,S83A) and parC(S80L,E84K).If the incorrect antimicrobial agents are chosen to treat A.baumannii infection, the outcome of patients may be poor. Thus, a rapid assessment of antimicrobial susceptibility could have a significant impact on patient care. The amplification refractory mutation system (ARMS), is a PCR-based method of detecting single base mutations. The purpose of this study was to screen clinical isolates of Acinetobacter baumannii for these mutations associated with fluoroquinolone resistance by the ARMS-PCR analysis.

Methods: A.baumannii isolates (n = 120) originated from three hospital settings in Tehran, shiraz and Gonbad were used in this study. These isolates were obtained from different Intensive Care Units(ICU) in the hospitals and were identified by using API 20NE system. Quality control strain used in antimicrobial susceptibility testing (Acinetobacter baumannii[ATCC 19606]) were purchased. The antimicrobial susceptibility of the clinical isolates were determined using the broth micro dilution protocols of the Clinical and Laboratory Standards Institute (CLSI) against fluoroquinolones antibiotic. Analysis of the mutations in the genes gyrA (S83L,S83A) and parC(S80L,E84K) was performed by ARMS-PCR analysis.

Results: Assessment of antimicrobial susceptibility patterns indicated that most isolates revealed a high rate of resistance (>80%) to fluoroquinolones. The mutation rate in gyrA (S83L,S83A) and parC (S80L,E84K) were 80% and 40%,respectively.

Conclusion: Resistance to fluoroquinolones is mainly related to the acquisition of point mutations in the sequence of the gyrA and parC genes. ARMS PCR technique is cheap, precise and very rapid technique for mutation analysis; therefore, in clinical center such as hospital where diagnosis should be carried out rapidly, and for detection of common gyrA and parC mutations is a preferable technique.

Keywords: Acinetobacter baumannii, gyrA and parC Mutation, ARMS-PCR analysis

P522: **Coincidence of common bacterial isolates with similar antimicrobial susceptibility patterns among contaminated medical foods and patients' clinical samples in a teaching hospital in Tehran, Iran.**

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Background and Aim: Hospital acquired infectious diseases are still a major healthcare problem in the world. Some percentage of these infections can cause through ingestion of contaminated foods in hospitals. The aim of this study was to investigate role of hospital foods in transition of clinically important bacteria into the hospital environment.

Methods: A total of 322 samples from hospital's personnel, patients' clinical and environmental samples, and 210 samples from the hospital kitchen, including raw and cooked foods, food handlers and food processing devices were cultured on Blood agar and Mannitol salt agar plates. Swab culture and aerobic plate count assay were used for isolation of suspected bacteria. Colony count of each culture and biochemical identification of each isolate was determined according to standard methods. Antimicrobial susceptibility of each bacterial isolate was determined according to the latest clinical laboratory standard guideline.

Results: A total of five different bacteria were isolated from 210 food samples, of which the most common was Staphylococcus aureus (15.23%), followed by Escherichia coli (8.09%), Enterococcus spp., Pseudomonas spp. (0.47%) and Acinetobacter (2.38%). Among the ICU samples, S. aureus (8.7%), E. coli (1.55%), Enterococcus spp. (3.1%), Pseudomonas spp. (1.24%) and Acinetobacter spp. (3.41%) were identified. 11.66% of the S. aureus isolates from utensils', food staffs' and hospital personnels' samples, showed same resistance pattern (cefoxitin, cefepime, amoxicilin clavulanic acid, penicillin and cefoxitin, amoxicilin clavulanic acid, penicillin). Multidrug resistant isolates of E. coli were also detected in both of food and clinical samples. Contamination of food staffs' samples with vancomycin resistant Enterococcus spp. was seen in 100% of the isolates.

Conclusion: Outbreaks of foodborne disease in hospitals are reporting in developing and developed countries. Frequency of clinically important bactera in studied hospital foods and their processing units emphasis control of their dissemination into hospital environment.

Keywords: Hospital acquired infection- foodborne disease- antimicrobial susceptibility

P523: **Antimicrobial Drug Resistance profile of nosocomial bacterial infection agents isolated from two general educational hospitals of Tehran, Iran**

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Background and Aim: Nowadays a rapidly growing crisis in antimicrobial resistance is happening, especially among microorganisms that cause nosocomial infections. Nosocomial infections are associated with a great deal of morbidity, mortality and increased financial burden. Resistance to antimicrobial agents is a major problem worldwide, Knowledge of the pattern of antibiotic resistant microorganisms in hospitals is essential for the effective empirical treatment and reducing complications and cost. The main objective of this study is to study the epidemiology and antimicrobial drug resistance of the most commonly isolated bacterial strains from two hospitals in Tehran.

Methods: In this survey we defined 404 samples of hospitalized patients who were referred to educational hospitals of university of Tehran, Iran. Samples were collected from 138 wound, 40 sputum, 63 blood, 30 cerebrospinal fluid (CSF), 78 urine, 55 tracheal tube specimens and cultured them on standard medium culture environments. The isolated strains were identified by differentiative tests and underwent susceptibility testing according to CLSI guidelines (Kirby-Bauer disk diffusion technique). Patient's data such as gender and age were recorded. We used SPSS, version 16 for data analysis.

Results: out of 404 patients, 254 patients 124 (%49) male and 130 (%51) female were positive in culture. Among nosocomial pathogens, Acinetobacter spp (30%) was the most frequently pathogen followed by Staphylococcus aureous 21%, klebsiella spp (10%) , Escherichia coli (7 %) and Pseudomonas spp (14 %). The most common isolated strains from lung , urinary tract, surgical site tissue and bloodstream were Acinetobacter baumannii, and Pseudomonas aeroginosa , Escherichia coli and Klebsiella pneumoniae, Staphylococcus aureus and Enterobacter spp respectively. Acinetobacter baumanni showed resistance to Cefotaxime 100% , to imipenem 86% and demonstrated the most sensivity to colistin. Pseudomonas aeroginosa was resistance to carbapenem 68% , to ceftazidime 75%. Klebsiella pneumoniae was the resistance to ceftriaxone 100% and carbapenem 41%.

Conclusion: This study showed a high percentage of resistance to antimicrobial agents in Pseudomonas aeruginosa and Acinetobacter baumanni isolates, therefore strategies to control the spreading of multidrug-resistant strains have to be designed and developed. In addition, new therapeutic regimes are clearly needed. High antibiotic resistance in all studied bacteria was showed that alarms an emerging public-health concern. It needs developing new antimicrobial agents and using of appropriate susceptibility testing Methods before therapy.

Keywords: Antimicrobial Drug Resistance , nosocomial infections, multidrug-resistant

P524: **Antibacterial activity of extracts from some of Iranian traditional medicinal herbs against coagulase-negative staphylococci**

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Background and Aim: Nowadays the side effect of antibiotics is a major problem for the health care systems. Due to increasing use of antibiotics, there would be crucial to find a safe, effective and more harmless replacement for them. This study tried to find a safe and secure treatment for the pathogenic infections which has caused by coagulase-negative staphylococci. In order to reach this goal, the extracts from some of Iranian traditional medicinal herbs such as Dracocephalum polychaetum, Myrtus communis, Thymus vulgaris and Eucalyptus camaldulensis were screened for the potential antibacterial activity against coagulase-negative staphylococci isolated from clinical samples at Afzalipour Medical Centre of Kerman.

Methods: In this in-vitro study, 56 samples have been taken from clinical patients, which had been collected from their blood, skin, longs and other part of the body that were infected and then were tested by Gram Staining, Catalase, Coagulase and fermentation of Mannitol on MSA (Mannitol Salt Agar) tests to isolate coagulase-negative staphylococci. Agar disk diffusion method was used for both antibacterial activity of herbs' extracts and antibiotics. The dilutions of herbs' extracts were provided with DMSO (Dimethyl sulfoxide). Blank discs were prepared at concentration of 30 mg/ml then tested against the samples. Two common used antibiotics which were Oxacillin (1 µg/disc) and Cefoxitin (30 µg/disc) used as positive control.

Results: From all 56 samples, 17 samples were identified as coagulase-negative staphylococci. Of all extracts, Methnol and Ethanol extracts of M. communis and E. camaldulensis showed inhibitory activity against all samples while D. polychaetum and T. vulgaris extracts showed no inhibitory activity. The average zone of inhibition for M. communis extract was 4mm and E. camaldulensis was 3mm while the average Cefoxitin zone of inhibition was 8mm and Oxacillin was 1mm.

Conclusion: Methnol and Ethanol extracts of M. communis and E. camaldulensis showed high inhibition against all samples, respectively. Their inhibition zone was more than Oxacillin and less than Cefoxitin. Hence, the isolation and the purification of therapeutic potential compounds from M. communis and E. camaldulensis extracts could be used as an effective and more harmless source against skin, blood, longs and other infections that caused by coagulase-negative staphylococci. It is suggested more in-vivo studies to make this as a goal.

Keywords: Dracocephalum polychaetum, Myrtus communis, Thymus vulgaris, Eucalyptus camaldulensis, coagulase negative staphylococci, antibacterial

P525: **The study of common SNP in FimH gene of Eschericia coli isolated from hospitalized and out-patient with urinary tract infections referring to educational Hospitals of Shahrekord**

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Background and Aim: Urinary tract infections (UTI) are one of the most common diseases of bacterial etiology occurring in human especially female sex. Different bacteria can cause UTI but the most frequently etiologic agent is uropathogenic Escherichia coli (UPEC) that possess type 1 fimbriae. Production of type 1 fimbriae is coded by fimH gene. Literature data emphasizes a significant role of type 1 fimbriae in UTI. Science the mutant alleles confer upon Escherichia coli a meaningfully higher tropism for uroepithelium; prevalence of C/T 640- SNP (single nucleotide polymorphism) was evaluated in this study.

Methods: For this reason, a total of 140 isolated Eschericia coli from patients with UTI were evaluated. Chromosomal DNA of bacteria extracted by boiling method and isolated E.coli strains were screened by PCR method for presence of FimH gene. Then strains with fimH gene were examined to detect C/T640 SNP by using RFLP method.

Results: Based on obtained results from PCR of 140 isolated strains, 130 samples (92/8%) had fimh gene. Results of RFLP-PCR showed among 130 strains, no strains possessing C/T640- SNP were identified.

Conclusion: Obtained results showed that prevalence of fimH gene in isolated Eschericia coli is in global range in Chaharmahal & Bakhtiari province. This absence of C/T 640- SNP is in accordance with some studies but in discrepancy with some others. Obtained results also showed that RFLP method could be served as a rapid, highly reproducible typing method for epidemiological studies of UPEC.

Keywords: Single nucleotide polymorphism (SNP), Urinary tract infection (UTI), Uropathogenic Escherichia coli (UPEC), FimH gene

P526: **Prevalence and antibiogram of methicillin resistant Staphylococcus aureus (MRSA) isolates isolated from personnel and clinical samples of Shahrekord teaching Hospitals**

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Background and Aim: Methicillin resistant Staphylococcus areus strains are major causative agents of community and nosocomial infections. The prevalence of these bacteria changes from country to country and hospital to hospital within a country. This study determines the prevalence of MRSA strains in the two important hospitals of Shahrekord and examines their susceptibility to major antibiotics.

Methods: A total of 262 samples including 244 nasal swabs from the personnel and 18 samples of patients referring to Hajar and Kashani hospitals of Sharekord were collected. The specimens were then cultured on mannitol salt agar for primary identification and isolation of Staphylococcus areus. The Staphylococcus areus isolates were identified using standard biochemical tests such as catalase, tube coagulase. Subesquently, in vitro antibiotic susceptibility tests of the isolates to oxacillin and other antibiotics were performed using growth on oxacillin screen agar and disc diffusion method according to CLSI guidelines.

Results: Prevalence of resistant to methicillin among Staphylococcus aureus strains were 13.4% (35 of 262). The results of antibiotic susceptibility tests showed that 33%, 23%, and 13% of (MRSA) isolates were also resistant to rifampin, teicoplanin and gentamicin, respectively. All strains were sensitive to quinopristin-dalphopristin, vancomycin and linezolid.

Conclusion: Despite the most recent studies, the prevalence of isolates that were resistant to methicillin and other antibiotics is low in this study and increasing resistant to methicillin is not a big problem in the mentioned hospitals. The most studies showed using the proper and continuous surveillance program is one the most factors affecting this issue that applied in the hospitals.

Keywords: Staphylococcus aureus, Methicillin resistance, methicillin resistant Staphylococcus aureus (MRSA), Shahrekord

P527: **Seroprevalence of Rubella Among Women in Childbearing Ages in Kashan During 2012-2013**

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Background and Aim: Rubella is an acute viral infection. There are disastrous malformations in fetus (congenital rubella syndrome) when pregnant women expose to the virus, especially in the first trimester. Diagnosis and vaccination of susceptible women is important. The objective of this study was to determine the prevalence of serological evidence of immunity to rubella among women in childbearing ages (14-36 years).

Methods: This study was conducted between March 2012 and March 2013. 1159 serum specimens were studied by the enzymelinked immunosorbent assay (ELISA) method. This study aimed to determine the level of protective antibodies against rubella among the 1159 women with age range of 14-36 who were referred to four laboratories in Kashan. Anti-Rubella IgG were measured in blood sera by ELISA kits (Pishtaz, Iran). According to antibody levels, test results were reported as immune and non-immune (susceptible) for every individual.

Results: Among 1159 cases, 19 women (1.63%) were non-immune (susceptible) and 1140 women (98.37%) were immune. The over all prevalence of seropositivity for IgG was 98.37% indicating that they were immune for rubella infection and the rest 1.63% were susceptible for rubella infection.

Conclusion: Rubella immunization rates in Kashan are not low and it was higher than the mean rate reported in Iran. Results indicated that immunity produced by the rubella vaccine is long-term. In conclusion, nearly 1.63% of women of reproductive ages are sensitive to rubella and should be vaccinated. We recommend vaccination of susceptible women before getting pregnant.

Keywords: Rubella, Immunity, ELISA, Kashan

P528: **Analysis of 1267G/A HSP70-2 gene polymorphism in gastric chronic inflammation in Iranian population**

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Background and Aim: Helicobacter pylori infection leads to long-lasting chronic inflammation and represents the most common risk factor underlying gastric cancer. HSP70-2 protein act as molecular chaperons in the folding of newly synthesized proteins in cells and assist in the refolding of damaged proteins. The HSP70-2 gene has a PstI site due to an A to G transition at the 1267 position and different genotypes of the HSP70-2 gene have been shown to be associated with a different level of HSP70 mRNA expression. It has recently shown that HSP70-2 polymorphism is associated with the risk of a certain subtype of gastric cancer. The purpose of this study was to investigate relation between polymorphism of the HSP70-2 gene (rs1061581) and gastric chronic inflammation because of H.pylori infection in Iranian population.

Methods: The study population consisted of 100 subjects. All subjects underwent upper gastroscopy. DNA was extracted from the biopsy samples and Genotypes were determined in patients and controls using PCR-RFLP.

Results: 50 subjects were diagnosed as gastric chronic inflammation and 50 subjects were diagnosed as normal subjects. Among cases, the distribution of genotypes was as follows: 15% was AA, 80% was AB, and 5% was BB. Among controls, the distribution was as follows: 40% was AA, 52% was AB, and 8% was BB.

Conclusion: The polymorphism of the HSP70-2 gene (rs1061581) is associated with gastric chronic inflammation. However, a larger clinical study should be undertaken with a larger population. Sample to investigate the real meaning of correlation between HSP70-2 polymorphism and gastric chronic inflammation.

Keywords: Gastric chronic inflammation, HSP70-2, Polymorphism

P529: **Minimal Inhibitory and Bacteriocidal Concentration Determination of Lactic acid bacteria Against Proteus Mirabilis**

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Background and Aim: The occurrence of multi-antibiotic resistance among Proteus mirabilis disease due to urinary tract infections is a prevalent clinical problem worldwide and continues to get serious due to the lack of efficient therapeutic options by the time. In this regards, antimicrobial compounds from Lactic acid bacteria with bactericidal or bacteriostatic activity looks one of the promising alternative to conventional antibiotics.

Methods: This study describes the determination of minimum inhibitory and bacteriocidal(MIC and MBC) concentrations for Lactobacillus acidophilus(ATCC 4356), Lactobacillus plantarum (ATCC 8014), Lactobacillus fermentum (PTCC 1638), Lactobacillus casei (PTCC 1608) and Lactobacillus rhamnosus (PTCC 1637) from Lactic acid bacteria against Proteus mirabilis (ATCC 7002) via the microtitre assay.

Results: L.acidophilus ,L.plantarum, L.casei, L.fermentum and L.rhamnosus showed a minimum inhibitory concentration of 25% , 25 % 25% 50% and 25% v/v and minimum bactericidal concentrations of 25% , 50%,50%, 50% and 50% v/v respectively.

Conclusion: The minimum bactericidal concentrations were discovered to be a 1 fold increase on those of the minimum inhibitory concentrations for L.plantarum, L.casei and L.rhamnosus. In addition, the data from comparative MIC showed the noticeable activity of selected potential Lactic acid bacteria strains against Proteus mirabilis (ATCC 7002). So the research highlights the potential use of Lactic acid bacteria for the clinical treatment of highly antibiotic resistant bacteria.

Keywords: minimum inhibitory concentration, minimum bacteriocidal concentration, Lactic acid bacteria , Proteus mirabilis

P530: **Prevalance of Mycolasma Hominis and Ureaplasma Urealyticum From Women With Abortion in Karaj's Hospitals.**

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Background and Aim: Mycoplasms constitute a large group of microorganisms, but only some, i.e. Mycoplasma and Ureaplasma species, are pathogenic for humans. The aim of the study was to assess the incidence of Ureaplasma urealyticum (U. urealyticum) and Mycoplasma hominis (M. hominis) infection in women with abortion who referred to Karaj's hospitals

Methods: M. hominis and U. urealyticum was assessed in 87 women with septic abortion with age of 18-50 years old who referred to Karaj's hospitals. A PPLO specific media (broth and agar) (Difco) by adding argenin(10%w/v) and urea(10%w/v) was used for determination of M. hominis and U. urealyticum respectively. Then SPSS-18 software is used for statistical analysis of data's.

Results: U. urealyticum was detected in 10 (11%), and M. hominis in 15 (17%) women. Patient with age 20-30 , in Second trimester period of pregnancy and with recurrent abortion showed most frequent to related infections.

Conclusion: The incidence rate of genitourinary infec¬tions due to M. hominis was significantly higher as compared to U. urealyticum infection in women with septic abortion in Karaj's hospitals. Sexual mycoplasmal infections were most frequently reported in the women with recurrent abortion and correlated with age and period of pregnancy.

Keywords: Mycoplasma hominis, Ureaplasma urealyticum, Septic abortion

P531: **Evaluation of the Humoral Immunity against Haemophilus influenza capsular polysaccharide (PRP)**

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Background and Aim: Haemophilus influenza type b (Hib) is an important cause of bacterial meningitis among children younger than 5 years of age. It is estimated that the morbidity & mortality rate of this invasive disease is about 400000 deaths of young children each year. Since,T-cell independent reponses in infants are weak, therefore, the immature immune system of infants are more vulnerable to infection. Developed countries by vaccination, have decreased this disease significantly to 90%. The capsular polysaccharide of Hib (PRP), is composed of polyribosyl-ribitol-phosphate, and has a key role in pathogenesis. The type b capsule inhibits the initial binding of C3, thereby reducing the uptake by Phagocytic cells. In order to enter the CSF, it adheres to the BBB and translocates through or remains inside cellular tight junctions.

Methods: Haemophilus influenza type B PTCC1623 was cultivated in 10L of CY broth medium in fermentor, and purified by alcohol precipitation method, and by the addition of Cetavlon, and Hydroxyl Apatite. In order to evaluate the humoral immunity, 2 groups consisted of two white New Zealand rabbits were chosen. Group 1 was injected by 25µg of Pure PRP in 0.5 ml serum physiology, and group 2 was injected by the same content of PRP, with an additional adjuvant AL2(OH)3(PRP-adj), with a two-week interval, intermascularaly, respectively. The blood was drawn at days 0, 15, 30, 45. The sera were separated, in order to evaluate the bacterical activity of the serum by Serum Bactericidal Assay (SBA), and ELISA in 450nm.

Results: Haemophilus influenza type B PTCC1623 was cultivated in 10L of CY broth medium in fermentor, and purified by alcohol precipitation method, and by the addition of Cetavlon, and Hydroxyl Apatite. In order to evaluate the humoral immunity, 2 groups consisted of two white New Zealand rabbits were chosen. Group 1 was injected by 25µg of Pure PRP in 0.5 ml serum physiology, and group 2 was injected by the same content of PRP, with an additional adjuvant AL2(OH)3(PRP-adj), with a two-week interval, intermascularaly, respectively. The blood was drawn at days 0, 15, 30, 45. The sera were separated, in order to evaluate the bacterical activity of the serum by Serum Bactericidal Assay (SBA), and ELISA in 450nm.

Conclusion: According to the results obtained, the antibody titer against PRP+adj was significantly more than the antibody against Pure PRP, in both ELISA and SBA methods. antibody to the capsular polysaccharide of Hib mediates protective immunity in adults. Compared to children, Adults have highly effective T-Cell responses to this polysaccharide with adjuvants. Since, the immature immune system of infants are vulnerable, the T-Cell independent response in children are weak. Therefore, PRP could be considered as a potential candidate to be used in future ELISA kits or to be used as a carrier in vaccine development for Hib.

Keywords: Haemophilus influenza type b, humoral immunity, poly saccharide, polyribosyl-ribitol-phosphate,

P532: **Detection of mrpH gene among Proteus mirabilis isolated from urinary tract infections as a new vaccine candidate**

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Background and Aim: Proteus mirabilis is a common cause of urinary tract infection (UTI) particularly in patients with catheterization and with abnormalities in the urinary tract. The prevalence of antimicrobial resistance in patients with UTI is increasing, making treatment of these infections ever more complicated and costly. Therefore there is a need for an efficacious vaccine against UTI. To date, no ideal vaccine against UTI has been approved for human use and there is a need to test other target antigens to develop an ideal vaccine against UTI. MrpH, the tip adhesin of MR/P fimbriae play an important role in pathogenicity of P. mirabilis. We evaluated the presence of mrpH gene in Iranian P. mirabilis isolates, confirmed by sequencing; in order to examine it as a new vaccine candidate against UTI cause by P. mirabilis.

Methods: Twenty Proteus mirabilis isolates were collected from urine sample of catheterized patients from hospitals in Tehran, Iran. All urine samples were cultured on blood agar and MacConkey agar for 24 h at 37°C. Bacterial identification was performed by routine culture method and biochemical tests. Genomic DNA from the samples was extracted using the Phenol& Chloroform method. PCR amplification of mrpH gene of the Proteus mirabilis isolates was performed by specific primers designed for this purpose. The PCR products were cloned into the pET28a vector and the selected recombinant plasmids were subjected to sequencing by universal primers. Then, the phenotypic expression of MrpH was evaluated by mannose-resistant hemagglutination test with human erythrocytes.

Results: The mrpH gene was amplified in all of the Proteus mirabilis isolates tested. Comparison of the mrpH sequences with sequences of mrpH gene in the GenBank showed significant homology (?98%) between the mrpH sequences from our P. mirabilis isolates and mrpH from P. mirabilis strains in GenBank. All of the P. mirabilis isolates were positive for mannose-resistant hemagglutination test.

Conclusion: Proteus mirabilis is a frequent cause of UTI and a significant source of morbidity and mortality. The previous studies showed that mrpH gene is conserved among P. mirabilis strains and our results confirmed the conservation of the gene and their sequences among Iranian P.mirabilis isolates. Thus we concluded that mrpH gene might be an ideal vaccine candidate for the prevention of P. mirabilis UTI in human. Expression and evaluation of the immune responses of the vaccine candidate in vivo and in vitro is under study.

Keywords: urinary tract infection, Proteus mirabilis, vaccine candidate, MrpH, hemagglutination

P533: **Susceptibility pattern of Ciprofloxacin and gyrA gen in Streptococcus pneumoniae strains**

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Background and Aim: S.pneumoniae is a major life threading worldwide pathogen. Concern over the emergence of multidrugresistant strains has led to the development of antipneumococcal fluoroquinolones, such as Ciprofloxacin. So, the aim of this study was to detect the sensitivity of S.pneumoniae strains isolated from clinical specimens by using broth microdilution and E-Test and confirmed it by PCR.

Methods: In this study, for broth microdilution method, 96 well microplate were used. Ciprofloxacin uploaded by different concentrations as a triple repeat testing. 200 µl of Mueller Hinton broth medium, containing 3% sheep blood was added to all wells. Then 50 µl of bacterial suspension equivalent to 0.5 McFarland standards were added to each test and positive control and also negative wells and were incubated for 20 hours. Then, 98 well microplates were scanned with an ELISA reader at a wavelength of 625 nm. Results were analyzed with the software SPSS version 16. In continue, in E-Test method, bacterial suspension equivalent to 0.5 McFarland standard was cultured on Mueller Hinton agar medium by using a sterile swab. Then E-Test strips of Ciprofloxacin were established. After incubation for 20 h, the inhibition zones were measured and the MICs were determined. For confirmation of results, PCR was used to amplify the gyrA gen.

Results: The results of susceptibility pattern of 50 S.pneumoniae strains isolated from Tehran's hospitals to Ciprofloxacin in both methods showed that 2 strains had MIC more than 2µg/ml. as a result, 4% of the isolated strains were completely resistant. Moreover a 2100 bp band was detected in resistant strains by PCR.

Conclusion: The results of determining the resistant of 50 strains isolated from clinical samples to Ciprofloxacin showed that fortunately, resistance to this antibiotic is still low and this antibiotic is a proper option in treatment of pneumococcal infections. However, in order to prevent resistance to this antibiotic and other antibiotics, their consumption should be controlled.

Keywords: Ciprofloxacin, S.pneumoniae, E-Test and broth microdilution

P534: **GyrA and parC genes mutations in quinolone resistant clinical isolates of Pseudomonas aeruginosa from northwest of Iran**

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Background and Aim: Pseudomonas aeruginosa is one of the most important causes of nosocomial infections, especially in patients who are immunocompromised. During the past decade the production of anti-Pseudomonas agents has not progressed much while the resistance to existing drugs has increasingly been developed. Many Different antibiotics including fluoroquinolone are used for treatment of infections caused by this microorganism. However resistance to this class of antibiotics has been reported in recent years. Mutations in the gyrA and parC genes are important factors involved in the resistance to fluoroquinolone. In the present study, the frequency of mutations in gyrA and parC genes and the impact of these mutations on resistance to ciprofloxacin, the most active fluoroquinolone against Pseudomonas aeruginosa, have been investigated.

Methods: Ninety seven strains of P. aeruginosa were isolated from patients in Imam Reza Hospital in Tabriz. Antibiotic resistance was examined by disk agar diffusion after confirmational tests. DNA was extracted from colonies by simple boiling method. The gyrA and parC genes were amplified by PCR, and mutations in these genes were analyzed by RFLP method.

Results: According to the results of this study, gyrA gene mutations were detected in 67 strains of P. aeruginosa and all of them were resistant to ciprofloxacin. In 16 isolates mutation occurred in both gyrA and parC gened and all of them were resistant to ciprofloxacin. In 18 strains mutations was observed only in the parC gene.

Conclusion: The results of this study indicate that mutations in gyrA and parC genes may lead to resistance of P. aeruginosa to ciprofloxacin. Also mutation in GyrA gene showed more association with resistance to ciprofloxacin than parC gene.

Keywords: Pseudomonas aeruginosa, gyrA gene, parC gene, fluoroquinolones, ciprofloxacin

P535: **Frequency and antimicrobial resistance of enteroaggrigative Escherichia coli from young children with and without diarrhea in Zanjan**

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Background and Aim: Enteroaggregative Escherichia coli is one of the pathogenic pathotypes of E.coli in the digestive tract that lead to acute and chronic diarrhea in childrens. Most of the genes encoding the virulence factors as pcvd432 are located in the 60-65 MDa plasmid and also used for detection of the EAEC infections. The aim of this study was to investigate the frequency of EAEC and their antimicrobial resistance profile in children younger than five years with and without diarrhea.

Methods: In this cross-sectional study, Between 2012 and 2013, a total of 550 stool specimens from children younger than five years of age (400 with and 150 without diarrhea) were investigated for EAEC. After culture and verifying of isolatets by biochemical tests, Antibiotic susceptibility testing was performed by disk diffusion method as recommended by CLSI to 13 antibiotics. In order to detect EAEC in diarrheal and control samples, a primer pairs specific for PCVD432 gene was used in PCR.

Results: A total of 400 children with diarrhea and 150 control children without diarrhea were studied. 140 isolates of E. coli from diarrheal patients and 60 isolates from control group were collected. The frequency of EAEC isolated in diarrheal and control groups were 25.8% (36 isolates) and 8.3% (5 isolates), respectively. The most prevalent resistance profile was erythromycin (100%), followed by azteronam 80.7% (113 isolates) and amoxicillin 74.4% (104 isolates). Imipenem was found as an effectiveness antibiotic with susceptible rate 72.9%. Isolates were resistance to other antibiotics in following pattern: Amikacin (21.4%), Cefoxitine (32.1%), Cefotaxime (50.7%), Ceftazidime (62.8%), Co-amoxiclav (71.4%), Co-trimoxazole (21.5%), Ciprofloxacin (37.1%), Gentamicin (29.3%) and Tetracycline (69.3%). Also, 86.4% of isolates were resistant to three or more agents and considered Multidrug resistance (MDR).

Conclusion: The results show that the EAEC strains are the most common E. coli pathotype in the Zanjan. In addition, the frequency of antimicrobial resistance to various antibiotics was high in diarrheagenic E. coli strains. As a result more emphasis on the identification of these organisms is recommended.

Keywords: Enteroaggregative E.coli, Diarrhea, Children, Antibiotic resistance

P536: **Protective immune response against methicillin resistant Staphylococcus aureus in a murine model by a recombinant protein vaccine approach**

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Background and Aim: MRSA as a methicillin resistance S.aureus causes severe nosocomical and community acquired infections at various body sites, also leads to severe morbidity and mortality. Resistance of S. aureus to methicillin and all beta-lactam antibiotics is a result of a protein (ie, penicillin binding protein2a (PBP2a) that is found in the membrane of MRSA. PBP2a encoded by mecA gene located on the chromosome of MRSA that shows low affinity for beta-lactam antibiotics. Today, antibiotic therapy for MRSA infections is limited due to antibiotic resistance of the organism, and this has led to a focus on immunoprophylaxis by active and passive immunisation. The main aim of the present study was to develop a vaccination strategy based on recombinant PBP2a that would enhance the protective response against MRSA in the murine model.

Methods: We cloned an internal region from the PBP2a with transpeptidase activity (amino acids 370-451) into the pET24 a (+) vector. Following the preparation of recombinant PBP2a , In order to immunization of experimental groups, Balb/c mice were injected subcutaneously with 20µg of recombinant PBP2a three times with three weeks intervals. The sera of experimental groups were collected three weeks after last immunization and then specific antibodies were evaluated by enzyme linked immunosorbent assay (ELISA). The antibacterial effect of the recombinant vaccine was evaluated by intraperitoneal immunization and challenge with a sublethal dose of MRSA for 10 days in mice. After the challenge, the number of bacteria from kidneys immunized and non-immunized mice were determined.

Results: Cloning of mecA was confirmed by enzymatic digestion and sequencing. SDS-PAGE and western blot analysis showed that recombinant protein with molecular weight of 13 kDa is over expressed. In addition, high titer of specific antibody against PBP2a in vaccinated mice was developed as compared to control group ($P < 0.005$), and confirmed the immunogenicity of vaccine candidate. Kidneys from immunized mice had 1000 times less on bacteria than the Control group (PBS).

Conclusion: Our results indicate that the PBP2a recombinant vaccine induced specific antibodies against methicillin-resistant Staphylococcus aureus and can be used as a vaccine candidate for further study.

Keywords: Methicillin-resistant Staphylococcus aureus, PBP2a, recombinant vaccine

P537: **The Incidence of Chlamydia trachomatis Infections in Pregnant Women, Implementation for Syndromic Management**

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Background and Aim: We would like to demonstrate that screening tests will lead to a fall in the prevalence rate of Chlamydia trachomatis (Ct.) in the community. Rather seldom are, however, any information collected on whether or not it represents data from opportunistic screening studies and has the potential to augment public health programs designed to control the asymptomatic infection in at-risk population (1).

Methods: A multiplex PCR test was offered to pregnant women aged more than 18 years old after consenting inform. Sociodemographic characteristics, sexual behavior, and clinical data were collected according to a standardized questionnaire. Subjects treated with antibiotics within the prior 3 weeks before sampling, were excluded from the study.

Results: Among 215 women attended in screening venue, 203 eligible females were included. The incidence of Chlamydial infection was 8.8%, and most infected patients were asymptomatic. Higher prevalence of infection was found in patients aged 20 to 30 years old. Statistical analysis showed significant differences of Ct. infection with: age <30 years old ($P<0.05$) and having had any level of educational degree ($P<0.01$).

Conclusion: In two previous studies which conducted on 2010 and 2011, we report a decrease in Ct. prevalence from 14.79% to 12.28% respectively (2, 3), and finally to 8.8% in this study (2012). In conclusion, based on our findings in this area, implementation of effective programs like systematic screening of at-risk groups with relatively high incidence of Sexually Transmitted Infections (STIs) are needed in Iran to identify and treat infections, especially among pregnant adults or those repeatedly infected, and particularly those at risk without symptoms.

Keywords: Chlamydia trachomatis, Pregnant Women, Management

P538: **Antimicrobial susceptibility of with respiratory infections of pseudomonas aeruginosa in Imam Reza General hospital , Tabriz 2011-2012**

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Background and Aim: pseudomonas aeruginosa is a virulent opportunistic pathogen that is one of the major causes of hospital acquired infection. It has the unique ability to infect bady systems. Despite introduction of a wide variety of antimicrobial agents with anti-pseudomonal activity is important.life-threatening infections caused by pseudomonas aeruginosa contribute to morbidity and mortality in hospitalized patient. Aim: aim from this study was conducted to determine the antibiotic susceptibility patterns of pseudomonas aeruginosa isolated from respiratory tract specimens obtained from hospitalized patients

Methods: 100 case pseudomonas aeruginosa gathered from the respiratory system of hospitalized patients during one years then they were tested for antimicrobial susceptibility using disk diffusion method.

Results: the rate of antimicrobial susceptibility of isolates were 81.5% to Imipenem, 79.8% to Amikacin , 55.3% to ciprofloxacin ,33.2% to Tobramycin ,25% to carbenicillin, 15.6% to ceftriaxone,10.8% to tetracycline,13.7% to cotrimoxozole, 5% to Cefexim.

Conclusion: Imipenem was the most effective and Amikacin was the secondary effective antimicrobial agent in this study. considering of antimicrobial resistance rate , surveillance of antibiotics therapy is necessary.

Keywords: Nosocomial Infection , Pseudomonas Aeruginosa ,Tabriz

P539: **The antibacterial activity of methanolic extract of Otostegia persica against Staphyloccoccus spp isolated from Sina hospital of Tehran**

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Background and Aim: Since bacterial drug resistance against common chemical drugs is increasing, researchers always are looking for new drugs with natural origin and less complications. In this research we studied the antibacterial effect of total methanolic extract of Otostegia persica against clinical Staphylococcus spp isolated from patients of Sina hospital of Tehran.

Methods: During June 2012, the plant of Otostegia persica was collected from Sistan and Baluchestan province (Zahedan). They were identified, dried, grinded and extracted by perculator method and using methanol. Based on standard bacteriologic methods, 80 Staphylococcus spp were isolated and identified from different clinical samples of patients of Sina hospital of Tehran during 2012. The antibacterial effect of methanolic extract was evaluated by broth micro-dilution method and MIC determined in comparison to amoxicillin. Simultaneously, Staphylococcus aureus PTCC1112 and Staphylococcus epidermidis PTCC1114 were used as standard control.

Results: Of 80 Staphylococcus spp were collected in this study the species frequency were, 71% S.aureus, 15% S.epidermidis and 14% other types (like S.saprophyticus). Also, samples were collected from blood cultures (50%), wound secretions(31%), tracheal tubes(9%). Moreover, 60% of samples were from infectious department and 40% related to ICU section. Based on microdilution method, MIC of total methanolic extract was determined 50 mg/ml in comparison to amoxicillin MIC which was 100 mg/ml.Among 80 tested isolates, 37% were resistant and 63% were sensitive. In addition of 63% sensitive isolates 40% was related to S.aureus and 23% to S.epidermidis.

Conclusion: With regard to obtained MIC(50 mg/ml to 100mg/ml), it seems the methanol total extract of plant Otostegia persica is two times more effective against standard and clinical samples of staphylococcus spp in comparison to amoxicillin. Besides, there were samples which were sensitive to methanolic extract of O.persica but resistant to amoxicillin. With attention to satisfied results, further evaluation tests like cytotoxicity survey is recommended for further studies.

Keywords: MIC, Otostegia persica, Staphylococcus aureus, Staphylococcus epidermidis, amoxicillin

P540: **Distribution of TEM, SHV, and CTX- M genes among gram negative non-fermenter isolates from burnt patients of Motahari hospital of Tehran**

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Background and Aim: Background and Aims: The acquired resistance against the wide-spectrum and highly stable beta-lactams is continuously increasing among gram negative hospital isolate. There resistant enzymes usually codified by genes associated with mobile genetic elements, are matter of major concern with regard to the future of antimicrobial chemotherapy because of its remarkable dissemination capability. The vast majority of them especially the ESBLs(Extended spectrum beta-lactamase) belong to the TEM, SHV, and CTX-M types. Hence, this study was performed to investigate the distribution of TEM, SHV, and CTX- M genes among nonfermenters as P. aeruginosa and A.baumannii. isolates from burnt patients of Motahari hospital of Tehran.

Methods: During 4 months (April 2012 to July 2012)75 non-fermenter isolates were collected from burnt patient of Motahari hospital, Tehran. All isolates were identified by conventional microbiological methods. Their sensitivity to common antibiotics were determined by disk diffusion method and ESBL screening was done by phenotypic DDST(double disk synergy test) by putting ceftazidim disk (30 µg)/ceftazidime clavulanic (30 µg/10 µg) and cefotaxime (30 µg)/cefotaxime clavlanic (30 µg/10 µg)disk in 20 mm distance, respectively based on CLSI 2012 protocol. ESBL positive isolates were used to identify the frequency of blaTEM, blaSHVand blaCTX-M by PCR method.

Results: Of 75 non fermenter isolates during 2012, 47) 63 (%P.aeruginosa and 28(37%) A. baumannii were identified. 26(34.6%) (15 P.aeruginosa and 6 A. baumannii) of the 75 isolates were confirmed as potentially ESBL producers by DDST test. Distribution of ESBL genes were as follows: 13% (2 from 15) TEM, 33% (5 from 15) SHV and 20% (3from 15) CTX-M in P.aeruginosa isolates and 33% (2 from 6) TEM, 16% (1from 6) SHV in A.baumannii. CTX-M not found among A.baumannii isolates.

Conclusion: ESBL positive strains of P. aeruginosa and A. baumannii are increasingly found in hospital isolates. Their high ability to pass the resistant genes to other clinical strains, need their quick detection in clinical laboratories. Further survey is needed among A.baumannii strains to detect resistant genes other than CTX-M.

Keywords: ESBL,Pseudomonas aeruginosa,Acinetobacter baumannii,SHV,TEM,CTX-M

P541: **Relation determining among Iranian Hp isolates and gastritis by PFGE.**

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Background and Aim: Gastric cancer (GC) is a common cancer among Asian countries including China and Iran. Despite of the lot of progress in radiology, endoscopy, adjuvant and non adjuvant therapy and surgery prognosis in endanger is less. Then just early prognosis and early treatment is the only way in GC patients to decrease their morbidity and mortality rate. However, their expensiveness and invasiveness limited their use. By new Olga staging, it was cleared that atrophyic gastritis mucosa is a sign of GC and its anatomic domain is related to cancer progress. In the other hand, genetic variation in Hp strains not only can increase their ability to cause different diseases but also possible their isolation by PFGE. And Finger printing methods. PFGE separation is 2 times more stronger than common electrophoresis method. The aim of this study was to determine any relation between Iranian Hp strains and gastric cancer stages by PFGE.

Methods: 80 biopsy samples ,were collected from patients who refered to Imam Hosain , Milad and Fayazbakhsh hospitals of Tehran.3 biopsy samples were collected from each patients for culture, rapid urease and pathology. All plates were incubated at micro-aerophilic condition and 37^0C.By PCR, existence of ureC and Helicobacter pylori (Hp) confirmation was done. Plag formation by Incert gold agarose and DNA digestion was done by NotI. Further PFGE was done in agarose 1% and heating at 50^0C. DNA fragments were visualized after ethidium bromide staining and under UV radiation.

Results: Biopsied were matched by pathology and Hp culture. Different PFGE profile were detected.

Conclusion: After analysis it was cleared that, there is no any genetic similarity between Iranian Hp strains and also the stage of gastritis.

Keywords: Helicobacter pylori, gasteritis, PFGE

P542: **Determination the frequency of blaIMP and blaVIM among nonfermenter isolates from burnt patients of Motahari hospital**

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Background and Aim: The emergence and rapid spread of metallo-β-lactamase (MBL)-producing isolates is a concern worldwide due to unsatisfactory empirical treatment. Since new metallo-β-lactamase (MBL)-encoding genes have been reported in the early 1990s from worldwide, emergence of MBL-encoding genes is worrying because of mobile nature and dissemination of the genes among Gram-negative nosocomial bacteria. MBL producing strains are often resistant to different classes of antimicrobial agents and associated with high rates of mortality, morbidity .So, for accessing to appropriate treatment and infection control and to prevent their dissemination, detection of MBL producing Gram-negative bacilli is necessary. Hence our study was undertaken to investigate the frequency of some metallo-beta-lactamases genes (blaVIM and blaIMP) in P.aeruginosa and Acinetobacter baummannii isolated from burnt patients admitted to burnt ward of Motahari hospital, Tehran.

Methods: During April to July 2012, 75 non-fermenter isolate were obtained from burnt patients who refer to Motahari hospital, Tehran. All isolates were identified by routine microbiological tests. Their antibiotic susceptibility to common antibiotics was done by disk diffusion method in accordance with CLSI 2012 standard guidelines. MBL producer among Imipenem (IMP) and meropenem resistant strains were screened by using meropenem / meropenem +EDTA (0.5M) in double disk synergy test (DDST). MBL positive isolates were further typed for existence of blaVIM and blaIMP by using PCR.

Results: Of 75 non fermenter isolates included, 47) 63 (%P.aeruginosa and 28(37%) A. baumannii were identified. 25 (13 P.aeruginosa and 12 A. baumannii) of the 75 isolates were confirmed as potentially MBL producers. By DDST, 7mm increasing in diameter of zone of inhibition in meropenem +EDTA(0.5M) disk vs. to meropenem disk alone were detected in 25(% 34.25)of detected strains (13 P.aeruginosa (17.8%) and 12 A. baumannii (16.4%)) . Distribution of MBL genes in isolates was as follow: 7.69% (1of 13) IMP in P.aeruginosa, 0% (not found) IMP in A. baumannii and VIM not found.

Conclusion: To prescribe suitable antimicrobial therapy and to prevent resistant dissemination early detection of MBL-producing organisms is crucial. Study of other MBL coding genes is recommended in further studies.

Keywords: MBL, PCR, P.aeruginosa, A.baummanii

P543: **Phenotypic survey of carbapenem resistant among E.coli strains isolated from patients of Imam Khomaini, Sina and Motahari hospitals of Tehran**

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Background and Aim: Nowadays, bacteria's resistance especially among E.coli strains against existing drugs has been increasing. One of the ways in which bacteria including E.coli acquire resistant against beta-lactams such as carbapanems, is through producing beta- lactamases. Some examples of these enzymes are MBL and KPC. Contamination with these bacteria increases the therapeutic costs and mortality. Since prescribing Carbapanem is the last chance for curing, so, it is critical to identify and screen them. Therefore, the aim of this study was phenotypic survey of carbapanem resistant among E.coli strains isolated from patients of Imam khomaini, Sina and Motehari hospitals.

Methods: 120 E.coli strains were collected from patients of Imam khomaini, Sina and Motehari hospitals during March 1390 to June 1392. All the strains were confirmed based on the standard methods of microbiology. Their sensitivity against common antibiotics was measured with disk diffusion and microdilution broth methods based on CLSI 2012 standards. The capability of MBL production was investigated with DDST using imipenem and meropenem alone and imipenem and meropenem+EDTA .The KPC evaluation was done by using ceftazidim and cefotoxin /bronic acid (Rosco diagnostica, Denmark) and Modified Hodge Test methods, respectively. E.coli ATCC25922 was used as control strain, simultaneously.

Results: Only one resistance strain to carbapanems was obtained from 120 collected E.coli isolates. This strain was resistance to Meropenem , Ertapenem and Imipenem. By microdilution broth method the MIC for Imipenem and Meropenem was 8 µg/ml and 64µg/ml respectively. Moreover, it was screened as MBL producer by using both Meropenem and Imipenem/ Imipenem EDTA after ≥7mm difference in resistant zone diameter .It was detected as KPC negative after using bronic acid and Modified Hodge Test.

Conclusion: Fortunately, the number of resistance E.coli strains to Carbapanems is so low (0.8%) in some Tehran's hospitals. However, doing antibiogram test is suggested before any antibiotic prescription to prevent bacterial resistance. Further molecular studies are in process.

Keywords: KPC, MBL, E.coli, imipenem, meropenem

P544: **Antibacterial activity of Thymus trautvetteri on Listeria monocytogenes isolated from red meat**

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Background and Aim: In traditional medicine, there are many natural crude drugs that have the potential to treat many diseases and disorders and one of them is Thymus. Thymus is a genus to the family Lamiaceae. Antibacterial activity of Thymus is related to thymol. The purpose of this investigation is to evaluate the susceptibility of L. monocytogenes strains isolated from red meat to antibacterial activity of Thymus trautvetteri . Penicillin was used as positive control.

Methods: The T. trautvetteri were collected from sabalan mountain area during spring. They were shade dried and pulverized in powder form. The powdered sample were extracted by soxhlet extraction method using chloroform. Then, solvents from extracts were removed under reduced pressure with the aid of rotary vaccum evaporator. The antibacterial test was done on Mueller Hinton agar medium (MHA) by spread plate technique. Mac Farland 0.5 concentration of both isolatet and standard strain (ATCC19114) of L. monocytogenes were used. In this study, also MIC was determined .

Results: Inhibition zones by crude extracts of T. trautvetteri were 12mm and 16mm for isolatet and standard strain respectively. MIC for isolatet listeria was 135µg/ml and 80µg/ml for standard strain. penicillin sensitivity in isolatet listeria was more than standard strain.

Conclusion: The increasing prevalence of multidrug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotic raises due to use of herbs for medicinal purpose is a universal phenomenon. Our results show that compounds of Thymus can be used for control of Listeria and food listeriosis. Further research can be conducted to isolate other potential activities of the T. trautvetteri.

Keywords: Thymus trautvetteri, Antibacterial, Listeria monocytogenes

P545: **Association of Interleukin-18 Gene Polymorphism with Visceral leishmaniasis in an Iranian Population**

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Background and Aim: Visceral leishmaniasis (VL) is a parasitic disease caused by a protozoan of Leishmania genus and in Iran by Leishmania infantum. Cytokines have a major role in determining progression and severity of clinical manifestations in VL. According to the important role of IL-18 in immunity against visceral leishmaniasis, this study was conducted to demonstrate the prevalence of genotypes on -656 G/T in promoter region of IL-18 gene.

Methods: This descriptive and cross-sectional study was done on 90 pateints with confirmed VL, 70 healthy seronegative controls and 129 seropositive controls. Salting out method was used to extract DNA and the amplification refractory mutation system (ARMS)-PCR procedure was used for detecting polymorphism at IL-18 -656 G/T.

Results: The frequency of IL-18 GT, GG and TT genotypes among all subjects were 24.2%, 15.6% and 31.1% respectively. According to the results, -656 T/T was the dominant genotype among the groups. Statistical analysis of distribution of genotypes was performed using Chi-Square test and reveal a significant difference among groups (P =0.044).

Conclusion: Polymorphisms of the IL-18 gene appear to play a major role in the genetic predisposition to VL in Iranian population.

Keywords: Interleukin-18, Visceral leishmaniasis, Polymorphism

P546: **Effect Q-solution at the ONE-STEP RT-PCR and DMSO at the conventional RT-PCR for replication region NS5b of HCV**

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Background and Aim: About 3% of the world population is infected with Hepatitis C virus (HCV) with total of about 180 million carriers. Sequential genes at the HCV are Core, E1, E2, p7, NS2, NS3, NS4a, NS4b, NS5a and NS5b. HCV NS5B codes for the viral RNA-dependent RNA polymerase. This section applies for detection and genotyping experimental. Furthermore Clinical studies had shown that mutation at some amino acid positions of NS5B occurred significantly common in the sustained virological responders group than in the non-responders group. NS5b gene is rich in GC content and secondary structure and thus PCR reaction for this portion is difficult. In this study, we adopted and facilities a PCR-based direct strategy for replications NS5b.

Methods: In this study both method conventional RT-PCR and ONE-STEP RT-PCR were done. But In order to facilitate and promote of the efficiency and remove nonspecific we have to use DMSO for conventional RT-PCR and Q-solution for one-step PCR.

Results: At the first without additive materials, amplification lead to products derived from regions other than the target DNA region that indicated by multiple bands on a stained agarose gel. But when DMSO and Q-solution added to conventional RT-PCR and ONE-STEP RT-PCR respectively, it brings about striking and sharp bands.

Conclusion: There are instances in which one-step RT-PCR and conventional RT-PCR conditions do not produce acceptable results. In those cases there are a number of additives for example DMSO and Q-solution that can be used to increase yield and specificity of the reaction.

Keywords: HCV NS5b, one-step RT-PCR, conventional RT-PCR, additive materials

P547: **Detection of qnr genes in ESBLs and non_ESBLs E.coli isolated from Clinical specimens of out-patient and hospitalized patients in Imam Reza Hospital,during 2011**

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Background and Aim: Escherichia coli (E. coli) are common bacteria that normally live in the intestine. Some of the E.coli strains produce extended-spectrum beta lactamase (ESBL) and are called ESBL-producing E.coli .these strains are resistant to cephalosporin antibiotics and a number of other classes of antibiotics .the aim of this study was to determine the Frequency of qnr genes in ESBLs and non_ESBLs E.coli isolated from Clinical specimens ofout-patient and hospitalized patients in Imam Reza Hospital ,during 2011.

Methods: Two hundred E.coli isolated from different clinical specimens. ESBLs producing E.coli were detected by determining their susceptibility to Ciprofloxacin and also by phenotypic confirmatory test. PCR assay was employed for detecting qnrA, qnrB, qnrC and bla TEM and bla SHV genes.

Results: Eighty six (43%) of isolates were Ciprofloxacin resistant . 85(42.5%) of 200 E.coli isolates were recognized as ESBLs producer by Phenotypic Confirmatory Test (PCT). The prevalence of bla TEM, blaSHV, qnrA, qnrB and qnrS genes were 65(76.47%), 23(27%), 63(31%), 34(17%) and 14(7%) respectively.

Conclusion: In our study, some organisms were isolated with both ESBLs and qnr genes, it should be as a result of insertion in same plasmid which can easily transmit to other organisms and increase the number of multi drug resistance isolates. This was first report of qnrS in E.coli producing ESBLs in Iran.

Keywords: E.coli, Antimicrobial resistance, ESBL, qnr

P548: **Sequence based typing and integron carriage in Acinetobacter baumanii: What is the merit?**

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Background and Aim: Acinetobacter baumannii is an important opportunistic pathogen responsible for a variety of nosocomial infections , especially in those admitted to burns and various ICUs. Since the organism also exhibit multiple-antibiotic resistance, it has been suggested that epidemic potential among isolates of A. baumannii may be linked to the presence of integrons. Several classes of integrons have been described, with class I integrons being the most common. Various molecular techniques have been involved for typing this organism and among these, sequence based one is the easiest, simplest, less time consuming, reproducible and cost effective technique, which involves Outer-membrane protein A (ompA) , csuE and Oxa66likes or Oxa 51likes. ompA is a porin and has also been found to induce apoptosis of epithelial cells. The csuE gene codes for part of a pilus assembly system, thought to be essential for biofilm formation. Many of the genotypes involved have been found to belong to pan-European clones (lineages) I, II or III. Thus, this study aimed at i) to determine the distribution of class 1, 2 and 3 integrons among these isolates, ii) to evaluate any correlations between antibiotic resistance and the carriage of different classes of integrons and iii) to type the isolates by sequence based method.

Methods: A total of 95 non-duplicate isolates of Acinetobacter baumanii were collected, confirmed as A. baumannii by conventional biochemical testing and later by PCR and later stored at –80°C for further investigations. Antimicrobial susceptibility testing was performed toward 19 antibiotics by disk agar diffusion and MIC (for ampicillin sulbactum, pipercillin, imipenem and meropenem) according to CLSI guidelines. PCR was carried out for class 1,2 and 3 integron. Multiplex PCR was carried out for sequence based type groups and the genes investigated for typing were ompA, csuE and blaOXA66-like.

Results: All 95 Acinetobacter isolates were confirmed by phenotypic and genotypic methods as A.baumanii . All isolates were resistant to the following antibiotics: ampicillin, cefazolin,and ticarcillin and the lowest resistance was found toward colistin (13.68%). PCR detecting integrase gene showed that 55.8% of all the isolates had intI 1; however, intI 2 was only identified in 15.8% of the isolates and intI 3 was not revealed in any of the clinical isolates. All isolates belonged to Group 1 , which corresponds to Group II European clone.

Conclusion: Our study shows multi –drug resistance A.baumanii strains associated with integron carriage, which could be a feature of epidemic clones and points to the necessity of a screening programme for patients, along with strict infection control regimes to prevent further antimicrobial-resistance selection and subsequent dissemination.

Keywords: Acinetobacter baumannii, Integron, Antibiotic resistance, Sequence based typing, Epidemics, Clone

P549: **Septicemia and antibiotic resistant pathogens in high risk patients: An ever increasing concern**

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Background and Aim: The number of patients admitted to intensive care units (ICUs), emergency and infectious diseases require more attention as compared to other wards in a hospital due to several reasons including severe underlying diseases, multiple operations, diagnostic and therapeutic invasive procedures which predispose critically ill patients to infection. Similarly in burn victims, invasion by the bacteria is not unexpected, despite advances in antibiotics. In view of immunocompromised condition, several published studies suggest carrying out periodic review of patterns of isolation and susceptibility profiles of microorganisms infecting such patients should be a routine in all burn and ICUs units. The aim of this study was to determine blood infections in the infectious diseases, ICUs and burn patients admitted to Sina Hospital, a teaching and referral center for Northwest Iran.

Methods: The study was carried out in patients admitted to burn, emergency and infectious diseases wards or referred to ICU including burns and infectious disease ICUs because of deteriorating condition. A total of 1503 set of blood samples (sent in blood culture bottles- Casteneda) were sent to Division of Microbiology where blood cultures were processed on blood agar plate after initial incubation for 24 hours on day 2nd as a “blind subculture” and at the last on day 5th before discarding specimen as negative. MacConkey agar plate was included if direct gram’s staining showed presence of a gram negative organism. The inoculated plates were incubated for 24 hours again, followed by isolation and identification of pathogens, in case blood culture was positive. All pathogens were analyzed for their antibiotic susceptibility pattern on Muellar Hinton agar by disk diffusion method according to CLSI. In case of fastidious organisms, Muellar Hinton agar was supplemented with blood and used for antibiogram. All negative specimens were discarded after 5 days of incubation.

Results: Ten percent of total blood specimens showed positive result , 32% septicemias were related to Gram positive bacteria, in an order from highest to low frequency: Staphylococcus aureus (42%), Enterococcus spp. (18%) and Staphylococcus epidermidis (16%). Causative agents in 62% of positive blood cultures included Gram negative bacteria, which in order of declining frequency included: Klebsiella pnumoniae (38%), E.coli (31%) followed by Pseudomonas aeruginosa (23%) and Acinetobacter spp. (3%). S.aureus isolates were susceptible to amikacin and vancomycin. Most of the Klebsiella pneumonia isolates were resistant to ciprofloxacin (75%) followed by ceftriaxone (62.5%) and gentamicin (60%). In contrast, 51.5% isolates of E.coli isolates were resistant to ciprofloxacin and 57% to ceftriaxone. The antibiotic pattern of Pseudomonas aeruginosa showed them to be resistant to ciprofloxacin (62.5%), imipenem (58.3%) , and ceftriaxone(54%). Acinetobacter spp. were found resistant to most of the antibiotics including carbapenems and aminoglycosides.

Conclusion: The surveillance conducted showed septicemia to be associated with multi –drug resistant pathogens. Since these microorganisms may be found everywhere in the hospitals, as effective control measures are required to limit their spread. Antibiotic policies of the hospital also require more attention.

Keywords: Septicemia, Pathogens, Antibiotic resistance , Surveillance

P550: **The relation between accessory gene regulator (agr) types of S.aureus and some phenotypic criteria**

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Background and Aim: The accessory gene regulator (agr) quorum sensing system of Staphylococcus aureus controls the expression of most of the genes encoding virulence factors and hemolysis. agr specificity groups is important because its relation to some type of diseases or phenotypic criteria. This study was carried out to determine any relation between agr specificity groups and antibiotics resistance, pigmentation and heamolysis in S. aureus isolated from Gorgan, North of Iran.

Methods: PCR-based assays were used to for the identification of agr specificity group and mecA presence in the 194 isolates of S.aureus. Other antibiotics resistances for S.aureus isolates were determined by Disc diffusion method, pigmentation on nutrient agar medium in 37 °C and 25 °C and hemolysis on sheep Blood agar medium were assessed.

Results: Except penicillin, in all of the antibiotics examined in this study, the isolates belonged to agr group III have the highest resistance to antibiotics. About penicillin the isolates belonged to agr group II have the highest resistance. The isolates belonged to agr group IV have greater ability to produce hemolysin (%60) and isolates belonged to agr group III have greater ability to produce pigment (%60.5).

Conclusion: The S.aureus isolates belonged to agr group III has more potential to acquire antibiotic resistance.

Keywords: S. aureus, agr group genes, drug resistance, hemolysin

P551: **Evaluation of Zataria multiflora Boiss and Carum copticum antibacterial activity on IMP-type metallo-beta-lactamase-producing Pseudomonas aeruginosa**

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Background and Aim: Carbapenem resistance due to acquired metallo-beta-lactamases (MBLs) is considered to be more serious than other resistance mechanisms .The aim of this study was to evaluate the antibacterial activity of Zataria multiflora Boiss and Carum copticum plants on IMP-producing P.aeruginosa strains.

Methods: This experimental study was carried out on hospitalized burnt patients during 2011 and 2012. Antibiotics and extracts susceptibility tests were performed by disc diffusion and broth microdilution methods. MBL detection was performed by Combination Disk Diffusion Test (CDDT) .The bla(VIM) and bla(IMP) genes were detected by PCR and sequencing methods

Results: Using Combination Disk Diffusion test method, it was found that among 83 imipenem resistant P.aeruginosa strains, 48 (57.9%) were MBL producers. PCR and Sequencing methods proved that these isolates were positive for blaIMP-1 genes, whereas none were positive for bla(VIM) genes. The mortality rate of hospitalized patients with MBL-producing Pseudomonas infection was 4/48 (8.3%). It was shown that Zataria multi?ora and Carum copticum extracts had a high antibacterial effect on regular and IMP-producing P.aeruginosa strains in 6.25 mg/ml concentration

Conclusion: The incidence of MBLs producing P.aeruginosa in burnt patients is very high. In our study, all MBL-producing isolates carry the blaIMP-1 gene. Therefore, detection of MBLs producing isolates are of great importance in identification of drug resistance patterns in P.aeruginosa and in prevention and control of infections. In this study, it was shown that extracts of Z.multiflora and C.copticum have high antibacterial effects on β-lactamase producer P.aeruginosa strains.

Keywords: P.aeruginosa; Metallo-β-lactamases; Zataria multiflora Boiss ; Carum copticum

P552: **Isolation and characterization Vibrio species from coastal waters of Bandar Abbas**

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Background and Aim: In medicine dictionary, the Vibrio word is a name for cholera disease. Vibrio species are gram-negative and comma shape that they have been known as the agent of digestive and extra intestinal disorders which usually appear epidemically in some seasons of the year. Identification and enumeration of pathogenic agents particularly pathogenic Vibrio is beneficial for control and prevention planning of the infectious diseases. As far as the fact that seas can be considered as one of the important ecosystems of Vibrio species, the aim of this study was prevalence review of Vibrio species in the coastal regions of south of the country in different seasons.

Methods: In this study, 600 taken water samples of coastal waters of Bandar Abbas were evaluated from point of Vibrio strains in both spring and winter. For primary detection of these strains, TCBS and alkaline peptone water were used, and subsequently the final identification was carried out by biochemical tests (oxidase, motility, Indole ring, KIA and other biochemical tests such as 0% NaCl, 6% NaCl, VP and ONPG).

Results: Statistical analysis of isolated samples showed that V. harveyi (2.3%), V. cholerae (1.7%), V. parahaemolyticus (1.3%), V. furnissii (1.2%), V. metschinikovii (1%), P. shigelloides (1%), A. hydrophila (0.5%), V. vulnificus (0.3%), V. mimicus (0.3%) and V. fluvialis (0.2%) have the highest frequency. Based on the studied seasons, 71.2% of pollution was related to winter and 28.8% to spring. Therefore, in winter the cases of pollution are more than spring.

Conclusion: separation of these species increases after precipitation and also in months that temperature of weather is more than 30 °C degrees. Base on the effect of this two parameters on the Vibrio species frequency, is mentionable that domestic wastewaters had more effect in this areas pollution and Vibrio species frequency decreased via repairing of coasts and control of domestic waste waters in spring.

Keywords: Vibrio species, intestinal diseases, coastal waters

P553: **Genetic diversity of Iranian clinical isolates of M. fortuitum**

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Background and Aim: The increase of infections caused by nontuberculous mycobacteria (NTM) is receiving increasing attention worldwide. Mycobacterium fortuitum is encountered with increasing frequency in clinical laboratories of Iran

Methods: Forty-eight M. fortuitum clinical isolates from different clinical samples between 2009-2012, were analyzed by sequence analysis for sequence variants within the 16S-23S intergenic sequence (ITS) region.

Results: Sequence analysis of the region in clinical isolates revealed seven different sequence types within ITS of M. fortuitum

Conclusion: The results shown that ITS sequencing is reliable for identification of M. fortuitum. This study also showed that the (ITS) region of the M. fortuitum exhibits a high variation and could be used to strain differentiation and typing.

Keywords: Mycobacterium fortuitum, ITS, diversity

P554: **The difference of expression recombinant MexR in E. coli TOP10 and E. coli BL21(DE3)**

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Background and Aim: Mina Hekmat doost, Mitra Salehi * Department of Microbiology, Faculty of Biological Science, North Tehran Branch, Islamic Azad University , Iran Pseudomonas aeruginosa is one of the most common pathogens involved in hospital infection causing opportunistic infections in humans, particularly among immunocompromised patients and resistance to antimicrobial agents makes treatment more difficult. In Pseudomonas aeruginosa, seven RND-type pumps have been described to date, although the major efflux determinant of intrinsic multidrug resistance and the best studied of these pumps in Pseudomonas aeruginosa is MexAB-OprM. The mexAB-oprM efflux operon is negatively regulated by MexR. The aim of this study was to compare the expression recombinant MexR from Pseudomonas aeruginosa in E. coli TOP 10 and E. coli BL21(DE3).

Methods: In this study, 104 multidrug resistant (MDR) strains, from different clinical sources, were isolated and identified through 16S rRNA sequencing and biochemical testing; and achieved a resistance pattern in 15 antibiotic discs through disk diffusion method and in 6 antibiotic discs through microdilution method. The genomic DNA was extracted. Then by using specific primers of mexR gene, it was amplified through PCR. The resulting mexR gene was cloned in pUC19 transformer for expression and the pUC19 plasmid was transferred to E. coli TOP 10 and E. coli BL21(DE3). After cloning was confirmed, MexR protein was extracted and its expression investigated by SDS-PAGE. Before and after cloning, antibiotic susceptibility pattern of E. coli TOP 10 and E. coli BL21(DE3) was tested, using disc diffusion assay and microdilution methods.

Results: The nucleotide sequence of PCR and mexR gene was totally the same. PCR products demonstrate the presence of mexR in 28 strains from 104 clinical isolates. The results indicated that recombinant bacteria became resistance to beta-lactams, fluoroquinolones, tetracycline and third generation of cefalosporine.

Conclusion: MexAB-OprM multidrug efflux system was expressed in only 27% of clinical isolates of Pseudomonas aeruginosa. Recombinant MexR can be produced by pUC19 in E. coli TOP 10 and E. coli BL21(DE3). The recombinant protein maintains its property desirably. The expression of MexR protein in E. coli BL21(DE3) was better than in E. coli TOP10.

Keywords: Pseudomonas aeruginosa, Multidrug Efflux Pumps, mexR, Cloning, Gene expression

P555: **The use of magnetic nanoparticles for DNA capturing and detection pathogenic agent**

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Background and Aim: Abstract The rapid detection of pathogenic agent is important especial in epidemic and bioterrorism .detection based on traditional method is costly and Tim long .the aim of this study is creative a rapid, specific, cheap and based on magnetic nanoparticle method for capturing DNA and detection bacterial with PCR

Methods: Material¬&method-aims: In this study, we tested a DNA extraction method, super paramagnetic nanoparticles conjugated to a DNA-capture sequence (probe) to a specific region of DNA of the genus bacterial. Then, a PCR assay was performed with primers specific for the genus bacterial to assess the specificity and sensitivity of the nucleic acid extraction method.

Results & Conclusion: Conclusions: The results suggest that the use of probe conjugated paramagnetic nanoparticles could be effective for the specific purification of microbial DNA in cultured or environmental samples, ensuring sensitivity and specificity of the subsequent PCR assays.

Keywords: magnetic nanoparticle,diagenostice,bacterial DNA.biotinalated prob

P556: **The use of Nano clays as detection of pathogenic agents**

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Background and Aim: Abstract Nanoclays are minerals that are at least one of their dimensions in the nanometer range, This is due to the cheapness and availability of materials, have attracted much attention in the field of nanotechnology¬ Being pure cationexchange capacity, two important characteristics for success as the strength of the polymer nanoclay to be considered Nanoclays because of their specific characteristics, in medical and pharmaceutical¬ industry have been considered. The clay as raw material in manufacturing of drugs and auxiliary substances used in drug synthesis.

Methods: Method and material: the montmorillonite due to the suspension properties, and mold release and having a good dispersion, much used in medicine as a supplement to good to be In this method of Nanoclays mentioned Venice silver nitrate solution, zinc and zinc sulfate with homogenization using And the ion exchange between Nanoclay and materials used and the layer being, it can be.

Results & Conclusion: Discussion and conclusions: The Antibiogram disk to Nanvkly ion isloaded with copper, silver and zinc and soaked in culture medium containing the staphylococcus bacteria, Pseudomonas, and Escherichia coli as well as empty disk was used as a control sample And results of the inhibition zone was investigated and reported. The team loaded up the broad-spectrum antibiotics in the nano-size is used for drug delivery purposes.

Keywords: Nanoclay, drug delivery, bacterial culture, Antibiogram, montmorillonite

P557: **Correlation between presence of psl operon and biofilm formation in burn isolates of Pseudomonas aeruginosa**

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Background and Aim: Pseudomonas aeruginosa is an important opportunistic pathogen in hospital acquired infections and responsible for high mortality rates in burn centers. The ability to form biofilms is a critical element for pathogenesis of Pseudomonas aeruginosa. Biofilm formation is mediated by polysaccharides which are the key components of the biofilm matrix. The psl operon encodes a putative polysaccharide that plays an important role in P. aeruginosa adhesion, critical for initiation and maintenance of biofilm structure. The aim of this study was to investigate presence of psl locus in relation with biofilm formation in burn isolates of Pseudomonas aeruginosa.

Methods: Sixty-two isolates of P. aeruginosa were obtained from burn patients at Shahid Motahari Hospital in Tehran. Biofilm formation was examined using the microtiter plate assay and detection of the pslA gene (the first gene in the psl gene cluster) was carried out using specific primers and PCR.

Results: Of the 62 test isolates, 27 (43.54%) formed biofilm by the microtiter plate assay, all of which also carried the psl gene. In addition, 5 isolates with biofilm negative phenotype harbored psl gene, which means that the expression of the pslA gene is influenced by other factors.

Conclusion: This study showed a strong association between psl gene carriage and biofilm formation, suggesting the importance of the psl gene in the ability to form biofilm in Pseudomonas areuginosa burn isolates.

Keywords: psl gene, biofilm, Pseudomonas aeruginosa

P558: **Comparison of Enzyme-Linked Immunosorbent Assay method (ELISA) with routine tests; VDRL , RPR for screening of serum infections with Treponema pallidum**

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Background and Aim: Background: Treponema pallidum (TP) is the causative agent of syphilis, a contagious and infectious systemic disease. Syphilis is classified as acquired that infection is usually transmitted by sexual intercourse or congenital by passing infection from mother to her fetus across the placenta during pregnancy. The incubation period of syphilis can vary from 1 to 13 weeks, but usually from 3 to 4 weeks. The risks of contagion are greatest during the first 2 years of infection. Two types of antibodies are produced by T.pallidum: nontreponemal antibodies(regain) and treponemal antibodies. The significant prevalence of syphilis in the world especially in developing country is increased from the year 2000 and became a serious problem, diagnosis the infection and then treatment is the best choise to control the disease. So detect a simple, fast, sensitive and specific method to diagnose infected sera is vital.

Methods: Method: Serology is still the most reliable method for laboratory diagnosis of syphilis. The mainstay of diagnosis for Treponema pallidum infection and treatment monitoring is based on nontreponemal and treponemal serololgic tests. Many new diagnostic methos for syphilis have been developed. Recently scientists have found that Enzyme Immunoassays are the best method to diagnose and confirm infection in patients. In this study we aimed to compare the diagnostic accuracy(sensitivity and specificity) of the RPR(Rapid Plasma Reagin) test, the VDRL(Venereal Disease Research Laboratory) test and an innovated ELISA kit for detecting total antibody in patient's sera. 510 samples which just 11 were positive, were tested by all 3 methods. Then after consideration of their reactivity, their sensitivity and specificity were compared to each other.

Results: Result: from 510 samples were tested with 3 methods, any false reactivity (positive or negative) were not seen by ELISA kit, but with two others methods, there were false positive and negative reactions. So the sensitivity and specificity of ELISA both were 100%. The sensitivity and specificity of RPR were respectively 90% and 97% and sensitivity and specificity of VDRL were respectively 90% and 94%.

Conclusion: Conclusion: we could introduce a new sandwich ELISA procedure to detect total antibodies against TP with the highest sensitivity and specificity (100%). comparison between 3 methods showed that this method is so useful to screening and treatment monitoring without any false reactivities.

Keywords: Treponema pallidum, RPR, VDRL, ELISA, syphilis

P559: **Rapid detection of UV-active antibacterial metabolites against MRSA of actinomycetes isolated from soils of Iran**

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Background and Aim: One of the most serious public health problems in the past decade is the rapid rise of antibiotic-resistant strains, particularly Methicillin-Resistant Staphylococcus aureus (MRSA) which is due to the broad use of antimicrobials in medicine, animal husbandry and agriculture. Therefore, finding new compounds against MRSA is among the urgent discovery programs in pharmaceutical industry. Actinomycetes are known for the production of different classes of therapeutic antibiotics including aminoglycosides, anthracyclins, glycopeptides,β-lactams, macrolides, nucleosides, peptides, polyenes, polyethers, terpenes and tetracyclines. Aim of this study was to find anti-MRSA secondary metabolites-producing actinomycetes through screening isolates from Nocardia, Kibbella, Sacchrothrix, Micromonospora and Streptomyces.

Methods: Isolates were selected and subjected for fermentation. After fermentation, extraction with solvent was done to obtain crude extracts of the selected strains. In other to visualize an almost complete picture of the secondary metabolites; Thin Layer Chromatography (TLC) was performed. TLC bioautographic overlay assay can help to rapid detection of the antimicrobial compounds against test strain. UV activity and chemical reactivity was used to select the strains for carrying the bioautography out. 4 investigated strains was selected for TLC bioautographic . In order to investigate anti-MRSA compounds, crude extract was fractionated using Preparative Thin-Layer Chromatography (PTLC).

Results: Among them strains UTMC951 and UTMC 728 showed strong activity and in addition, growth inhibition zone of crude extract of strain UTMC 951 covered UV-active metabolites.Five different semi pure fractions were obtained of crude extract from strain UTMC 951 by PTLC. TLC bioautographic of the fractions showed antimicrobial activity of 3 fractions against MRSA.

Conclusion: In this study a combination of chemical and biological approaches based on TLC technique was used in analysis of actinomycete metabolites for antibacterial compounds. Strains UTMC951 and UTMC728 are introduced as valuable candidates of promising antibacterial metabolites-producing strains against MRSA. Further investigations on active compounds of these two strains along with their chemical structure are on the way to be discovered.

Keywords: Actinomycetes, TLC Bioautographic antibacterial metabolites, MRSA, Thin Layer Chromatography

P560: **Prevalence of qnr genes in ESBLs and none-ESBLs Klebsiella pneumoniae isolated from Mashhad, Iran.**

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Background and Aim: Fluoroquinolones broad-spectrum of activity widely used by physicians to be led to the increase in resistance to it among bacteria, especially in Enterobacteriaceae. In this study, qnrA, qnrB and qnrS, new plasmid-mediated quinolone resistance genes, were detected among ESBL-Positive and ESBL-Negative Klebsiella pneumoniae .

Methods: One hundred and thirty samples of K. pneumonia were collected from Imam Reza Hospital and its associated clinic from May 2011 to July 2012. They tested for production of ESBLs by conventional method. PCR method was performed for detection of qnr A, B and S genes.

Results: Among 130 patients with K. pneumonia infection, 56(43%) produce ESBL, 17.86% (n=10), 30.36% (n=17) and 46.42% (n=26) K. pneumonia producing ESBLs were positive for qnrA, qnrS and qnrB, respectively. Four samples (%7.14) were negative for qnr genes.

Conclusion: Our study showed that qnr genes could accompany with blaTEM and blaSHV genes. In Klebsiella pneumoniae, quinolone resistance has been found more frequently in strains producing plasmid-mediated ESBL (37.5%) than in ESBL-negative strains (20.89%).

Keywords: Klebsiella pneumoniae, Quinolones, ESBL (Extended-spectrum B-lactamases)

P561: **Strains of E. coli are a serious risk in the communities of East Azarbaijan effected by earthquake**

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Background and Aim:: Nowadays, The main concern in earthquake affected areas is outbreaks of water and food-borne communicable diseases due to the carcasses of dead animals and lack of access to health facilities. These factors increases the possible outbreaks of infectious diseases such as diarrhea and intestinal diseases. E. coli bacteria among other species has a more upwardpath for infection.These bacteria live in our gut and it is the biggest cause of urinary tract infections. It is has the second largest population and is considered as an indicator for municipal sewage pollution.The aim of this study was to compare various strains of E. coli contamination in the stricken communities in East Azerbaijan province

Methods: In this study, 125 randomized samples of stool collected on two occasions from the residents of 12 villages in earthquake effected area and then they cultured in a specific medium and evaluating the overall test E. Coli strains were identified.

Results: Of the samples, 36 samples infected with Escherichia coli (28.8%) were diagnosed which from this population, 15 patients is effected with strains (ETEC) Enterotoxigenic(41.67%) and 20 patients is effected by strains (EHEC) Enterohemorrhagic(55.55%) and one patient is effected by strains (EIEC) Enteroinvasive(2.78%).

Conclusion: Due to the considerable variation in the rate of infection in different strains of bacteria in the stricken communities in East Azerbaijan province as a pilot, accurate analysis seems necessary to identifydifferent strains and also use of molecular techniques is required for accurate identification of strains.

Keywords: Escherichia coli, earthquake, pollution

P562: **determine prevalence of Listeria monocytogenes in aborted featus of sheep in kazeron**

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Background and Aim: Listeria monocytogenes is widely distributed in the environment and present the soil and the faeces of healthy animals. Bacterial pathogens were the most prevalent cause of abortion. Listeria monocytogenes is one of rare infective pathogens that could transferred via placenta during pregnancy and cause still birth or abortion in ruminants. Disaster of listeria is it could not produced and prevalent signs in pregnant animals. The most important cause of infective abortion of sheep and cow are infective and contagious and zoonosis between human and animals. That they are very important in public health .the aim of this study was to determine prevalence of Listeria monocytogenes in aborted featus of sheep in kazeron

Methods: The sheep fetus samples were collected from farms in kazeron during autumn to winter of 2011 (n = 50). Then abomasumal content and tissues of aborted fetus of sheep were prepared by autopsy. Samples were subjected to microbiological and antibiotic screening test. Samples of abomasumal content and tissues enriched by cold enrichment method and cultured on blood agar and listeria selective agar and confirmed by biochemical test MRVP, catalase, hemolytic and cAMP activity.antibiotic screening tests were carried out on Muller- Hilton agar and antibiotic disc method. Results were evaluated with standard strain of PTCC1163

Results: The contamination rate of L. monocytogenes was 36% (18/50), others bacteria 56% (28/50) and non contaminated 8% (4/50), respectively. The contamination rate of L. monocytogenes was 36% (18/50), others bacteria 56% (28/50) and non contaminated 8% (4/50).All isolated Listerial colony were detected sensitive to ampicillin and tetracycline and resistant to penicillin and erythromycin. Standard control strain was shown same results

Conclusion: Our results were shown relative contamination to listeria monocytogemnes in aborted sheep fetus that could be a public health concern

Keywords: • Abortion;sheep ; Listeria monocytogenes;fetus

P563: **Anti-tuberculosis activity of some medicinal plants used in traditional medicine in North of Iran**

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Background and Aim: The emergenc multiple-drug resistant (MDR) strains MDR-TB, resistant to rifampicin and isoniazid,and Extensively-drug resistant (XDR) strains of M. tuberculosis, resistant to both first and second-line drugs , Has essential the search for new drugs appropriately The present study was done to evaluate in vitro anti-tubercular activity of three medicinal plants on the growth of Mycobacterium tuberculosis.

Methods: Three hydroalcoholic extract of medicinal plants , Harmala peganum seed, meat of Citrus limetta and fruit of Berberis vulgaris that Were isolated from their natural habitats in Gorgan, northern Iran , prepared by maceration method. For each extracts, appropriate concentrations from 200 to.1.5 mg / ml was prepared. Anti-tuberculosis activity of this extracts on eight of Mycobacterium tuberculosis, isolated from patients and One H37RV strain were examined by Diffusion Method Disk. two strains were MDR and the others were sensitive to isoniazid and rifampin. Diameter of inhibition zone more than 10 mm was considered as a significant inhibitory effect.

Results: hydroalcoholic extract of Harmala peganum showed good inhibitory effect on the 9 isolates of M. tuberculosis and in concentration of 200 mg / mL, inhibition zone diameter was evaluated 22 mm. Berberis vulgaris fruit extract showed a weak inhibitory effect , Just on a sensitive isolate (Diameter of inhibition zone 10 mm). Citrus limetta extract had no inhibitory effect on any of MDR M.tuberculosis isolates But showed a significant inhibitory effect (more than 12 mm Diameter of inhibition zone) on two sensitive isolates

Conclusion: hydroalcoholic extract of Harmala peganum showed good inhibitory effect on MDR and non-MDR M.tuberculosis isolates. It is recommended that in the next studies, the effect of Harmala peganum extract will be investigated in cell culture models and animal models.

Keywords: Herbal extract, Mycobacterium tuberculosis, inhibition effect, Harmala peganum

P564: **CCR5-Δ32 mutation is not association with the outcome of hepatitis B virus (HBV) infection in Iranian population**

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Background and Aim: Hepatitis B is a serious and prevalent disease in the world and host genetic factors are an important factor in the progression of the disease. CCR5 is a main chemokine receptor on the surface of more immune cells and CCR5-Δ32 is a functionally null allele containing a 32-bp deletion. Some studies reported that there is a relationship between CCR5-Δ32 with clearance of hepatitis B in some of the world's populations. The aim of this study was to investigate association between this mutation with hepatitis B in Iranian population.

Methods: A total of 200 blood samples including 100 healthy controls and 100 HBsAg positive patients were randomly selected. Samples were tested for HBsAg by ELISA and HBV-DNA by PCR method. Genomic DNA was extracted from blood buffy coat using salting out method and the CCR5-Δ32 mutation was genotyped by PCR with specific primers. Chi-square test was used for statistical analysis.

Results: Results of this study showed that none of the subjects in the control and patient groups had CCR5-?32 mutation; therefore there was no difference in genotypes frequency of CCR5-?32 between controls and HBV infected patients.

Conclusion: It seems that CCR5-Δ32 polymorphism is not associated with HBV infection outcome in Iranian population.

Keywords: CCR5; hepatitis B disease; Δ32 mutation

P565: **Evaluation of antimicrobial property of propolis extract against pathogenic bacteria**

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Background and Aim: Propolis is a specific compound produced by bees. This compound derived from plant resin which assembled by bees. The purpose of this study was evaluation of the antibacterial effect of Propolis extract against pathogenic microorganisms and determines its active compound.

Methods: Propolis was obtained from several honey bee farms in kazeroun city in season spring and extracted by several solvents such as distilled water, methanol 70%, ethanol 70%, acetone and chloroform. Then the effect of extracts was evaluated against pathogenic bacteria viz., Bacillus cereus, Salmonella typhi, E-coli, Staphylococcus aureus and pseudomonas aeruginosa. Finally, active compound of propolis was determined by several chemical tests.

Results: The results obtained indicated that the best solvent relatively was methanol and this extract showed antimicrobial effect against all the bacteria tested. Out of all bacteria Bacillus cereus and pseudomonas aeruginosa were more and less sensitive respectively. Furthermore chemical tests carried out on propolis extract showed that the active compound of propolis probably is flavonoid.

Conclusion: Overall, propolis could be considered special compound with antibacterial property , which produced by bees.

Keywords: propolis extract -antimicrobial property-methanol extract-flavonoids compounds

P566: **Molecular characterization of virulence factors ,pertactin and fimbriae, in Bordetella pertussis strains isolated from clinical samples in Iran**

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Background and Aim: Bordetella pertussis is a causative agent of pertussis (whooping cough) in humans. Pertactin and fimbriae of B. pertussis are considered as important virulence factors in these bacteria. Pertactin is a 69-KD protein which has been identified on the surface of this pathogen and is a major protective agent of B. pertussis. Fimbriae or pillus antigens of B. pertussis have been shown to be one of the many adhesions present on the surface of the bacteria.In the present study, we amplified and sequenced prn and fim genes as important virulence factors of B. pertussis strains in order to study polymorphism of these genes in circulating bacteria in the communication in in compare with standard strains in the future.

Methods: We examined 39 culture positive samples isolated from nasopharyngeal samples collected in 2008-2012. These strains have also been identified by biochemical tests. Regions of prn and fim genes in these isolates of B. pertussis were amplified using specific primers by PCR method.

Results: our results showed that all examined strains in this study have fim and prn genes in their genome in size 800 bp and 600 bp, respectively.

Conclusion: In this study all strains isolated which confirmed through culture had both of virulence factors fim and prn that given the importance of role of these genes in immunization against B. pertussis. In consider with polymorphism in the sequence of the studied genes in B. pertussis and the effect of such genetic variation on immune response of the body to vaccine, further investigations about the Polymorphisms could help us to find predominant alleles of the virulence factors in communication.

Keywords: Bordetella pertussis-virulence factors-pertactin-fimbria-

P567: **Purification of Brucella abortus strain RB51 outer membrane proteins by sodium Lauroylsarcosinate method and protein expression by electrophoresis assay and its immunogenicity in laboratory animals**

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Background and Aim: Malta fever or brucellosis is considered as one of the most important zoonotic diseases. Brucella affects wide range of domestic and wild mammals. In recent years the use of live vaccineslike abortus strain S19, melitensis strain RevI and other vaccines have been prevalent to control brucellosis melitensis, but the vaccines sometimes cause abortion. Thereforewe should find vaccines with high immunogenicity and low complication, in this regard Brucella outer membrane proteins (OMP) as immunogenic structures can be used to design and produce a vaccine for brucellosis. However, there are different methods for OMP extraction with advantages and disadvantages.For this experiment we used Sodium Lauroylsarcosinate, according to the amount of extracted protein using extraction chromatography and electrophoresis. And Serum bactericidal assay is performedto determine the immunogenicity.

Methods: The bacterial OMP were extractedby sodium laurylsarcosine method. Fractions of OMP purified by the Lauroylsarcosinate method, whichhave been passed from 100 tubes through the sepharose chromatography column on 4B-CL,were collected and the absorbance at 260 nm UV was measured. The OMP abortus was then performed in the SDS-PAGE assay. To perform SDS-PAGE assay OMP fraction less than 20 microliters should not be taken. For the measurement of serum bactericidal, we use two New Zealand white rabbits. Each rabbit was injected 50 mcg physiology serumcontaining 50 mcg OMP, made of Sodium Lauroylsarcosinate method with aluminum hydroxide, two times within 15 days. And the blood serum was separated by centrifugation and the serum bactericidal methods were evaluated.

Results: In SBA conclusion the protein efficiency was also noted that the results obtained from this method have high protein immunogenicity.

Conclusion: Lauroylsarcosinateis a method that breaks the cell with Ultrasound,so the LPS is sent outand the extracted OMP contains LPS and acid nucleic. With special techniques we measured LPS and acid nucleic poorly seen in Sodium Lauroylsarcosinate method but the efficiency of protein was high. In SBA conclusion the protein efficiency was also noted that the results obtained from this method have high protein immunogenicity.

Keywords: Sodium Lauroylsarcosinate -SDS-PAGE assay-serum bactericidal method

P568: **The relationship between urine culture results with urine analysis in diagnosis of children urinary tract infections in medical diagnostics laboratory of jahrom.**

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Background and Aim: A urinary tract infection is common in all age groups .Lack early diagnosis and treatment of the urinary tract that can cause severe complications such as Urinary tract disorders ,hypertension, uremia, preterm delivery in pregnant women and even cause abortion. The disease is higher in women than men and occasionally has been reported more than three times the risk of women to men. In this study,the relationship between urine culture results with urine analysis in diagnosis of urinary tract infections in medical diagnostics laboratory of jahrom was evaluated.

Methods: 1239 urine samples from patients referred to jahrom lab with symptomatic UTI was examined. This samples with method of “Clean voided midstream” was collection. then urine culture and urine analysis was done. Samples cultured directly by standard looped on the blood and Macconkey agar.it was kept for 24 hours at 37 C. The plates wich have more than 100 pure colony were considered positive and after identifying the antibiogram test was done to them. For urine analysis 10ml of samples were Centrifuges for five minutes with 2500 rpm. A drop of sediment were transferred to slides to determine the number of Leukocytes, erythrocytes and epithelial cells. Data were analyzed by SPSS software.

Results: From 1239 samples 942 culture positive 731 of cases women and 211 were men. The most common age was 23-31 years and the most common bacterium E.coli. antibiogram test showed the Amikacin had highest and Ampicillin lowest sensitivity. Urine analysis from culture-positive cases indicated 730 cases included WBC, 208 cases RBC, 103 cases sugar, 88 cases protein, 42 cases cylinder and 421 cases crystal.

Conclusion: Results showed that the women was high risk more than men for urinary tract infections. Leukocytes, crystals and protein in the urine of patients with positive urine culture significantly higher than negative samples but a significant number of samples with positive urine culture were no above identified factors in urine analysis. Although there is a relationship between some parameters of urine analysis and bacterial urine ،but clinical symptoms and the presence of leukocytes in urine alone does not confirm infection. For proper treatment of patients and to prevent excessive consumption of antibiotics is necessary analysis, urine culture and antibiogram were be used simultaneously.

Keywords: Urinary Tract Infection- urine culture- urine analysis- Laboratory of Jahrom

P569: **Therapeutic effects of Nigella Sativa in rats infected with Staphylococcus aureus resistant to Methicillin.**

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Background and Aim: Infections caused by S.aureus resistant to methicillin(MRSA) mainly are with origin of hospital,in the world are on the rise.For this reason,researchers have many efforts to find new compounds as alternative for these antibiotics. Due to increasing resistance S.areus than other antibiotics such as erythromycin, tetracycline and even vancomycin and widespread disease caused by S.areus such as endocarditis, osteomyelitis, pneumonia, toxic shock syndrome.Led to the continuous efforts to find new antimicrobial drugs.One source of such drugs,its plants that are in rich and traditional medicine of Iran is used to experimentally. Antimicrobial effects of black seed by studies in which this herb caused increases immune cells and monocytes somewhat proven. Therefore, this study aimed to investigate the therapeutic effects of Nigella sativa in rats infected with MRSA was done.

Methods: Nigella Sativa after prepared and approved by the relevant experts then become powder form and feeding rats with standard doses 0.01 ,0.1and1g/kg body weight of rats was mixed. 40 Male rats a 10-8 Week and Wistar strain and similar conditions weight,in standard temperature,and good nutrition were maintained selected for the study,0.5 ml of bacterial suspension MRSA wich isolated from the wounds of hospital patients and its resistance to methicillin was confirmed by differential tests containing 1 million bacteria were infected through injected into the rats tail and then randomly divided into 4groups, three groups received the same doses and control group that received normal diet were divided. Before testing, for counted monocytes from the rat tail blood samples was performed. After4 weeks, from rats heart 3ml blood sample was taken a few drops use for peripheral blood smear to count monocytes and 2 ml were used in real time pcr to measure the microbial load.

Results: Percentage of monocytes before and after bacteri inoculation, respectively in the control group 2.9±0.2% and 4.3±1.1%,In the receptor dose 0.01 g / kg of black seed powder 2.5±1.2% and 4.9±1.0%, In the receptor dose of 0.1 g / kg of black seed powder3.01±0.3% and 5.1±0.8%,In the receptor dose 1g / kg of black seed powder 2.8±2.0% and 6.1±1.9% was calculated. Microbial load in first time injecting bacteri in all groups was 1 million MRSA. After 4weeks of microbial load in control group 4.45×106, In the receptor dose 0.01 g / kg of black seed powder3.82×106, In the receptor dose of 0.1 g / kg of black seed powder2.63×106,In the receptor dose 1 g / kg of black seed powder2.01×106,bacteri were calculated.

Conclusion: The results indicate Nigella Sativa have a positive impact on the number of monocytes. Note that the microbial load in the control group who have not received any dose of Nigella greatly increased but with given and increasing doses of Nigella sativa we observed a significant reduction in microbial load.Therefore receive and increasing doses of Nigella sativa also is significantly increased the number of monocytes and reduction in microbial load($P<0.05$).Hopefully will be more and wider research can use this plant complement or even replacement one of the drugs against MRSA.

Keywords: Black Seeds - Staphylococcus aureus - methicillin - resistant - arranged - antibacterial

P570: **s synovial fluids′A Laboratory study on rheumatoid arthritis patient**

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Background and Aim: Etiology of rheumatoid arthritis (RA) is not clear. Some reports indicated that micro organisms are involved in the disease. Probably one or more common factor had been involved. The most common of these factors are super antigens. The aim of this study was to investigate the presence of bacterial super antigens by using bacteriological methods, PCR and ELISA in synovial fluid samples from patients with rheumatoid arthritis.

Methods: during one year of a cross-sectional study, the selected patients referred to the rheumatology clinic 85 synovial samples were collected in a volume of 10 to 15 ml. 5ml of them was inoculate to Castaneda medium, the rest of the micro-tubes of 2 ml were collected for molecular analysis and ELISA. After incubation microbial cultures using the physical and biochemical characteristics were identified. Also, each of the samples were taken separately DNA extraction and PCR was performed using specific primers. These samples were analyzed by ELISA. The data were grouped and analyzed.

Results: During 12 months, 85 patients with rheumatoid arthritis were included. In a aseptic condition Castaneda medium were follow up for 2 weeks. The results of Sub culture revealed only 3isolated bacteri. Two Gram-positive coccies (possibly Staphylococcus) and gram-negative bacillus was diagnosed. Resulting molecular diagnosis of genetic enterotoxin A & B in some prepared Broth Media were positive and by ELISA was confirmed.

Conclusion: The results indicate that existence of super antigens in synovial fluid of RA patients. While, there are unknown site of origin. Samples that were isolated had shown the presence of the genes of super antigens which their express were confirmed by Elisa. However, further research will be necessary.

Keywords:: PCR, ELISA, Rheumatoid arthritis, superantigens, Bacterial culture and Synovial fluid

P571: **Antimicrobial Resistance Patterns of Pseudomonas aeruginosa clinical isolates in Hamadan city**

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Background and Aim: The opportunistic pathogen Pseudomonas aeruginosa is noted not only for its metabolic versatility and its exceptional capacity to adapt to and colonise a wide variety of ecological environments (water, soil, rhizosphere, animals), but also for its intrinsic resistance to a broad range of antimicrobial agents. Treatment of infectious diseases becomes more challenging with each passing year. This is especially true for infections caused by the opportunistic pathogen Pseudomonas aeruginosa, with its ability to rapidly develop resistance to multiple classes of antibiotics. The objective of our study is to describe the resistance pattern of clinical isolates of P. aeruginosa strains in Hamadan city.

Methods: A total of 100 P. aeruginosa clinical isolates were studied from three major hospitals in Hamadan city During July to December 2012. Antimicrobial susceptibility to 16 antibiotics was determined according to the Clinical and Laboratory Standards Institute (CLSI) using the Kirby-Bauer disk diffusion assay on Mueller-Hinton agar. The minimal inhibitory concentrations (MIC) of carbapenems were determined for the isolates by E-test.

Results: Resistance was most often observed to Ampicillin/Sulbactam (96%), followed by Cefotaxime (67%), Ciprofloxacin (38%), and Carbenicillin (30%), and to a lesser extent Gentamicin (28%), Levofloxacin (27%), Tobramycin (27%), Piperacillin (26%), Ceftazidime (24%), Cefepime (22%), Meropenem (22%), Imipenem (20%). The most Sensitivity was observed to Piperacillin/Tazobactam (81%), Amikacin (81%) Aztreonam (77%) and Colistin Sulphate (96%). A total of 25 isolates that resistance to Imipenem and Meropenem, 24 isolates showed high resistance (MIC>32 µg/ml) and one isolates had an intermediate resistance (MIC=12 µg/ml) to Imipenem by E-test.

Conclusion: Our results could reflect some hospital multidrug resistant Pseudomonas aeruginosa strains in nosocomial infections. According to this results, resistance to carbapenems seem not to be low among P. aeruginosa isolates from three hospitals of hamadan (25%). Since Carbapenem antibiotics are often used as the last line of treatment for infections caused by resistant P. aeruginosa, this could be an increasing concern for treatment.

Keywords: Pseudomonas aeruginosa, Antimicrobial Resistance, clinical isolates

P572: **Photodynamic bactericidal efficacy of hypericin and mucolytic agent against Pseudomonas aeruginosa in Biofilm Culture**

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Background and Aim: The rise of antimicrobial resistance is compounded by the inappropriate use of antibiotics (e.g. for viral infections), non-compliance of patients, and widespread antibiotic use in animal husbandry. Bacteria growing in biofilms are much more resistant to antibiotics. Pseudomonas aeruginosa is one of the opportunistic pathogens commonly found in wound infections and has been known to form biofilm easily and quickly .It produces high levels of quorum sensing molecules and alginate which produced by mucoid Pseudomonas aeruginosa infections protect both biofilms and the P. aeruginosa microcolonies by restricting antibiotic diffusion . acetylcystein(AC) is a non-antibiotic drug. It is a mucolytic agent that disrupts disulphide bonds in mucus and reduces the viscosity of secretions. AC is widely used in medical practice via inhalation, oral and intravenous routs and has an excellent safety profile. AC was found to decreas biofiml formation of variety of bacteria nad reduces the production of extracellular polysaccharide matrix while promoting the disruption of mature biofilms. Since the susceptibility of biofilm-associated bacteria is enhanced in disrupted biofilms. Antimicrobial Photodynamic therapy (aPDT) employs a non-toxic dye, termed a photosensitizer (PS), and low intensity visible light which, in the presence of oxygen, combine to produce cytotoxic species. aPDT is an emerging alternative to treat infections . Hypericin(HYP) is a natural photosensitizers found in Hypericum perforatum plants, commonly known as St. John's wort .

Methods: five biofilm-producing multidrug-resistant P. aeruginosa, isolated from chronic wounds, were used in this study. For biofilm eradication studies, pre-formed biofilms coincubation with acetylcystein (7 mg/ml) and HYP(0.5 µg/ml) and irradiated with LED with wave-length of 590±10nm for 10 min(Radiation dose 16 J/cm2). colony were counted after incubation for 24h at 370C.

Results: It was observed that the bactericidal effect of aPDT on pre-formed biofilms of P. aeruginosa strains, was significantly higher than that observed for control groups (viable count reduction of 99.99% for P. aeruginosa strains growing as biofilms). Acetylcystein was found to increase the therapeutic efficacy of aPDT by degrading the extracellular polysaccharide matrix of biofilms.

Conclusion: These data suggest that aPDT/acetylcystein combinations have the highest ability to eradicate pre-formed mature biofilms. Although anti-biofilm agents themselves might not kill the bacteria, they can make them more susceptible to conventional antibiotics as well as to the action of the host immune system.

Keywords: Pseudomonas aeruginosa biofilm , antimicrobial Photodynamic therapy Hypericin , mucolytic agent

P573: **Photodynamic bactericidal efficacy of hypericin on Staphylococcus aureus Biofilms treated with mucolytic agent**

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Background and Aim: The rise of antimicrobial resistance is compounded by the inappropriate use of antibiotics (e.g. for viral infections), non-compliance of patients, and widespread antibiotic use in animal husbandry. Bacteria growing in biofilms are much more resistant to antibiotics. Staphylococci are important causes of nosocomial and medical-device-related infections. Their virulence is attributed to the elaboration of biofilms that protect the organisms from immune system clearance and to increased resistance to phagocytosis and antibiotics . acetylcystein(AC) is a non-antibiotic drug. It is a mucolytic agent that disrupts disulphide bonds in mucus and reduces the viscosity of secretions. AC is widely used in medical practice via inhalation, oral and intravenous routs and has an excellent safety profile. AC was found to decreas biofiml formation of variety of bacteria nad reduces the production of extracellular polysaccharide matrix while promoting the disruption of mature biofilms. Since the susceptibility of biofilm-associated bacteria is enhanced in disrupted biofilms. Antimicrobial Photodynamic therapy (aPDT) employs a non-toxic dye, termed a photosensitizer (PS), and low intensity visible light which, in the presence of oxygen, combine to produce cytotoxic species. aPDT is an emerging alternative to treat infections . Hypericin(HYP) is a natural photosensitizers found in Hypericum perforatum plants, commonly known as St. John's wort . The aim of this study was to evaluate the possible photodynamic inactivation effect of HYP combined with biofilm-specific agent such as acetylcysteine on pre-formed mature biofilms. Ten strains of (MRSA) were used in this study.

Methods: Ten biofilm-producing methicillin-resistant Staphylococcus aureus, were used in this study. For biofilm eradication studies, after treatment of pre-formed biofilms with acetylcystein (10 mg/ml), biofilms were exposed to HYP(0.5 μg/ml)and irradiated with LED with wave-length of 590±10nm for 10 min(Radiation dose 16 J/cm2). colony were counted after incubation for 24h at 370C.

Results: It was observed that the bactericidal effect of aPDT on pre-formed biofilms of S. aureus strains which were treated with acetylcystein, was significantly higher than that observed for control groups (viable count reduction of 99.99% for S. aureus strains growing as biofilms)

Conclusion: These data suggest that pre-formed biofilms treated with acetylcystein (disintegration of biofilm matrix) are more sensitive to aPDT compared to untreated biofilms

Keywords: Staphylococcus aureus biofilm , antimicrobial Photodynamic therapy Hypericin , mucolytic agent

P574: **comparison of effects of Citrus aurantium with nalidixic acid and nitrofurantoin and co-trimoxazol on urinary tract infections producer bacteria**

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Background and Aim: With using antibiotic in societies which usage of this kind of the drug without any limitation cause appear resistance against antibiotics.This survey consider to use the extract and essence of the citrus aurantium (which have a so many rate of planting in Iran) and also survey on extract on bacteria whose cause urinary tract infections, and compare this with common antibiotics priscribtion.

Methods: This study was experimental design. Flowers of plant were extracted with boil water by maceration method. The method of extraction of essence was hydrodistilation with clevenjer. Then we have been isolate the E.coli and Klebsiella, Staphylococcus Aureus, Staphylococcus Epidermidis, Streptococcus Agalactiae and Enterococcus Faecalis from UTI and then determine again this bacteria with subculture and put the exact diagnosis on them. Antibacterial effects of the herb extract, nalidixic acid and nitrofurantoin and Co-trimoxazol were evaluated by two methods of disc diffusion and MIC.

Results: Enterococcus Faecalis had 100% sensitivity in front of extract and Co-trimoxazol , and 90% against Nitrofurantoin , and 80% against Nalidixic acid. E.coli had 100% sensitivity against Co-trimoxazol, Nalidixic acid and 90% on Nitrofurantoin and it was totally resistance to extract and essence. Klebsiella Pneumonie had 90% to sensitivity to Nitrofurantoin, 80% to Co-trimoxazol, 75% to Nalidixic acid and resistance against extract and essence. Streptococcus Agalactiae was 100% sensitivity to essence and Co-trimoxazol and 90% against Nitrofurantoin and Nalidixic acid and shown 80% sensitivity against extract. Staphylococcus Aureus MRSA shown 100% sensitivity against Nitrofurantoin and Co-trimoxazol and 70% sensitivity against essence, extract and Nalidixic acid. Staphylococcus Epidermidis shown 100% sensitivity against Nitrofurantoin and Co- trimoxazol and 80% sensitivity against Nalidixic acid and 75% sensitivity against essence and extract. Extract and essence had inhibition effect on Enterococcus Faecalis and Streptococcus Agalactiae till 1/128 in MIC method.

Conclusion: after all we totally conclude that essence and extract of citrus aurantium have a much more effectiveness on gram positive bacteria compare to gram negative bacteria.

Keywords: essence and extract of citrus aurantium, gram positive and gram negative bacteria, Antibacterial, Antibiotic resistance, urinary tract infection.

P575: **Bacterial Urinary Tract infection and determination of their antibiotic resistance patterns in adults referred to the Farshchian hospital in Hamadan**

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Background and Aim: Background and Objective: Urinary tract infection (UTI) is one of the problems in the community and hospitals in all ages and both sexes. Many organisms are involved in UTIs. Antimicrobial resistances of bacterial pathogen are complaining treatment and recovery of infected individuals. The aim of this study was identification of bacterial UTIs and determination of their antibiotic resistance in Adult referred to the Farshchian hospital in Hamedan in 2011-12.

Methods: Methods: This cross – sectional study was performed on 214 individuals referred to the Farshchian hospital microbiology laboratory in 2011-12.The samples were cultured in blood agar and Macconkey agar. The identification of isolated bacteria was performed by Gram stain and biochemical tests. Antibiotic susceptibility tests were done with cotrimoxazole, nalidixic acid, gentamicin, ceftriaxone, nitrofurantoin, tobramycin, amoxicillin and ciprofloxacin disks by the Kirby –Bauer method.

Results: Results: We isolated 141 pathogens from 214 urine samples. Among of 141 patient, %54/2 and %45/7 were female and male ,respectively .The most common causes of infection were Escherichia coli(%61),Enterobacter(%10/7),Staphylococcus(%8/5),Pesudomonas aeruginosa (%7/9)and Proteus spp.(%7/1) .In total ,regardless of the type of bacteria ,highest resistance was to amoxicillin and lowest resistance was to nalidixic acid. Escherichia coli as the most common cause of infection had the same pattern in both sexes.

Conclusion: Conclusion: According to our study ,E. coli was the most common cause of urinary tract infection and the strains had highest and lowest resistance to amoxicillin and Nalidixic acid ,respectively.

Keywords: Keywords: Urinary tract infection ;Adults; Antibiotics; Antibiotic resistance

P576: **antimicrobial Effect of Gentamicin Solid lipid nanoparticule on Staphylococcus aureus**

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Background and Aim: solid lipid nanoparticles (SLNs) have been widely investigated as antimicrobial drug delivery platforms, of which several products have been introduced into pharmaceutical market. The main advantage of using these nanoparticles is their burst drug release profile attributable to their large surface area and drug entrapped onto the particles. They have higher drug entrapment efficacy therefore superior to liposomal systems. SLNs are typically particulate systems with mean diameters ranging from 50 nm up to 1000 nm for various drug delivery applications.

Methods: Solid lipid nanoparticles are particulate systems made from lipids, amount of melted lipids (39.5 mg) were dispersed in an aqueous surfactant contain gentamicin. SLN in this study were prepared by high speed homogenization and ultrasonic metod. Cholesterol were used as lipid materials and Tween 80 were used as surfactants. drug-loading efficiency was calculated by using the reverse method. Nanoparticles size Measure by nanozetasizer.

Results: The particle size was 282.3 nm and the drug-loading efficiency was 40%. Antibacterial effect on Staphylococcus aureus, in Mueller Hinton agar well diffusion method and MIC, MBC was evaluated. By increasing concentration of nano-drug antibacterial effect was increased. MIC equal to 2.6 µg/ml and MBC equal to 5.1 µg/ml. SLN without drug loading, hasnot antibacterial effect.

Conclusion: Even though the development history of SLN-based antimicrobial drug delivery systems is relatively shorter than other nanoparticle systems such as liposomes and polymeric nanoparticles, SLNs have shown great therapeutic potentials. The positive features of lipid nanoparticles leaded to the market introduction of many antimicrobial products.

Keywords: Solid lipid, nanoparticles, Cholesterol, Staphylococcus aureus

P577: **Evaluation of Antibiotic Susceptibility Pattern of Listeria monocytogenes PTCC 1297 (Serotype 4a) Exposed to Environmental Stressful Conditions**

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Background and Aim: Listeria monocytogenes is a frequent cause of bacteremia and central nervous system infections in adults, particularly in immune compromised patients. The objective of this study was to examine the viability and changes to antibiotic susceptibility of L. monocytogenes PTCC 1297 exposure to environmental stresses.

Methods: Cultures at exponential phase of Listeria monocytogenes PTCC 1297 (Serotype 4a) were subjected to sub-lethal environmental stresses such as ethanol (5%vol/vol), sodium chloride (% 7 wt/vol), acid (HCl, pH 5), hydrogen peroxide (600 ppm) and heat (45 ºC) in order to evaluate changes in viability counts and antibacterial susceptibility. Antibacterial susceptibility and minimal inhibitory concentrations (MICs) were determined by standard disk diffusion and broth dilution methods, respectively. After the stress treatments, viable counts were determined on listeria selective oxford agar, and survivor plots were constructed.

Results: Exposing L.monocytogenes PTCC 1297 to hydrogen peroxide (600 ppm) and heat (45?) significantly ($P < 0.05$) increased resistance to all selective antibiotics. But treating to stresses such as hydrochloric acid (pH 5), sodium chloride (% 7 wt/vol) and ethanol (5%vol/vol) increased sensitivity ($P < 0.05$) to the selected antibiotics.

Conclusion: The results suggest that adaptation to some stresses including hydrogen peroxide and heat increase resistance to antibiotics and this may interfere to chemotherapy against L. monocytogenes infections. This is while stresses such as ethanol, hydrochloric acid and sodium chloride act in adverse.

Keywords: Listeria monocytogenes, Drug resistance, Environmental stresses

P578: **Evaluation of H. pylori infection's effect on the prevalence of Giardiasis in children**

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Background and Aim: Helicobacter pylori is a common cause of chronic infection in humans. The infection has a worldwide distribution in all age groups; the most common chronic bacterial infection probably occurs in humans that almost half of the world's population is infected. Bacterial urease is able to convert urea to ammonia in the stomach that increases the stomach PH. In fact, pathogenic intestinal protozoa, especially Giardia lamblia earns opportunity passing through the stomach and cause disease. The aim of this study is to evaluate the effect of H. pylori infection on prevalence of Giardiasis in children.

Methods: During a year(1390) in Ilam city, H. pylori antigen was detected in stool samples of children (up to ten years old) by ELISA. Positive samples were analyzed for Giardia lambelia by direct smear method.

Results:: In total 20 positive cases for H.pylori infection, 6 cases of them (30%) were positive for Giardia lamblia.

Conclusion:: The results of this study suggest that H. pylori infection may provide favorable conditions for giardiasis infection but this presumption needs to further studies on more samples.

Keywords: Helicobacter pylori ,Giardia lambelia ,children

P579: **Prevalence of Resistance to Helicobacter pylori Strains to clarithromycin, metronidazole and amoxicillin in patients with gastrointestinal disorders in Isfahan city**

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Background and Aim: Background: Helicobacter pylori (H. pylori) is one of the most common infectious diseases in the world. It colonizes about 50-60% of the world's population. H. pylori resistance to antibiotics has become a global problem and an important factor in determining the outcome of treatment of infected patients. The purpose of this study was the determination of H. pylori resistance to clarithromycin, metronidazole and amoxicillin.

Methods: Materials and methods: A total of 78 strains of H. pylori were collected from 260 patients who were referred to Endoscopy Section of the Isfahan Hospitals from March 2011 to July 2012 with gastrointestinal symptoms Including gastritis, gastric ulcer, duodenal ulcer and gastric cancer and then we investigated the frequency of H. pylori resistance to clarithromycin, metronidazole and amoxicillin with the E-test and Modified Disk Diffusion Method (MDDM).

Results: Results: H. pylori resistance to clarithromycin, metronidazole and amoxicillin were 15.3%, 55.1% and 6.4% respectively

Conclusion: Conclusion: Information on antibiotic susceptibility profile plays an important role in empiric antibiotic treatment and management of refractive cases. H.pylori resistance to clarithromycin, metronidazole and amoxicillin in our study was upper than the previous studies in Isfahan. It is indicating that the rate of resistance to 3 antibiotics is increasing in Isfahan. Previous studies have indicate that H. pylori resistance may change with time even in the same population, However in order to prevent antibiotic resistance and to determine the most effective anti h. pylori regimen, continuous surveillances is needed. According to the results obtained in this study, using metronidazole in our region, Isfahan can lead to eradication failure in clinical therapies due to having highest rate of resistance but amoxicillin and clarithromycin prescribe for first and second lines of treatment against H. pylori.

Keywords: Helicobacter pylori, , Clarithromycin, Metronidazole, Amoxicillin

P580: **A comparative study of frequency prevalence of aminoglycoside modifying enzyme gene in Enterococcus feacalis and Enterococcus faecium isolated from patients referring to Imam Reza Hospital, Kermansh**

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Background and Aim: Enterococci cause different diseases in human and are important agents of nosocomial infections. Different antibiotics such as aminoglycosides are used for treatment of the infections caused by Enterococci. Nowadays Enterococcus infections are difficult to treat because of an increase in aminoglycoside resistant rate. Aminoglycoside resistance genes encode enzymes that change the antibiotic's structure. The aim of this study was determination of aminoglycoside modifying enzyme gene among Enterococci isolated from patients referring to Imam Reza hospital in Kermanshah 2009.

Methods: 138 clinical specimens collected from different wards of Imam Reza hospital were identified to the species level by biochemical tests. Antimicrobial susceptibility test against imipenem, tobramycin, kanamycin, erythromycin, ampicillin, ciprofloxacin and teicoplanin was determined by disk diffusion method. Minimum inhibitory concentration of gentamicin, streptomycin, kanamycin and amikacin was evaluated by micro broth dilution method. Aminoglycoside resistance genes aac(6')-aph(2") was analyzed by PCR.

Results: The prevalence of isolates were as follow; E.faecalis 45.7%, E.faecium 23.9%, the rest of the samples were non-bacterial. 89% of isolates were HLGR. Most strains showed MIC > 8192 ?g/ml against kanamycin, streptomycin and gentamicin. From 85 isolates 24.7 % (21/85) of E.faecium isolates and 50 % (43/85) of E.faecalis isolates carried aac(6')-aph(2").

Conclusion: Remarkable increase in incidence of aac(6')-aph(2") among HLGR isolates explain the relationship between this gene and high level resistance to aminoglycoside. As the resistant gene can be transferred between Enterococcus strains and increased prevalence of aminoglycoside resistance so using new generation of antibiotics is necessary.

Keywords: Enterococcus feacalis, Enterococcus faecium, Aminoglycoside resistance

P581: **Evaluation of antibiotic susceptibility pattern and performance of alcohol-based handrub in the staphylococci isolated from the neonatal intensive care units (NICUs)**

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Background and Aim: The hands normal flora of health care workers may serve as reservoirs for causing health care-associated infections in neonatal intensive care units (NICUs). The Staphylococcus ,especially coagulase-negative staphylococci (CoNS), which formerly regarded as harmless inhabitants of the skin and mucosal linings, are now recognized as a major cause of nosocomial infections in NICUs. Hand hygiene is considered the primary measure to reduce the transmission of nosocomial pathogens. In 2002, Centers for Disease Control and Prevention (CDC) revised the recommendations for hand hygiene to include the use of alcohol-based products for standard hand hygiene. However, the possible effects of hand hygiene products on the skin flora of personnel hands, including antibiotic resistance patterns, have not been extensively studied. The aim of this study was to assess antimicrobial resistance patterns of hand normal flora among nurses in NICUs and effectiveness of handrub on removing normal flora.

Methods: This cross- sectional study performed in a five-months period since November to March 2011 from all full-time volunteer nurses in 6 Teaching Hospitals in Shiraz. Among nurses, 48 (above 80%) agreed for participation. During the study, all the nurses were using the same alcohol-based hand hygiene product. The sampling from the nurses obtained in two steps with a sterile swab dipped in saline, one before washing hands with handrubs and the other was used after washing. After culture of samples, isolates were identified based on microbiologic standard tests. Disk diffusion test for antibiogram was performed by the CLSI standards.

Results: From swabs of nurses before using handrubs, a total of 42 CoNS isolates and 3 S. aureus were recovered. Also, 17 CoNS isolated from samples after using handrubs. Only 12 (25%) samples had positive culture after washing hands. Among 59 recovered isolates from nurses, 47 (79.6 %) were methicillin resistant coagulase negative staphylococci (MRCNS), in which contribution of 17 CoNS recovered after washing has been 70.5%. Results indicated a higher rate of multi-drug resistance from recovered isolates after using handrubs. Resistance to Erythromycin, Ampicillin and Clindamycin were 91.5%, 86.4% and 71.1%, respectively.

Conclusion: In conclusion, alcohol handrub eliminated Staphylococci in more than 75% of nurses' hand working in NICUs. Although, high resistance level resulting from antibiotic susceptibility testing of recovered isolates after washing indicated that alcohol-based handrub despite of decline in count of bacteria on the nurses hand failed in the elimination of multi-drug resistant strains.

Keywords: antibiotic susceptibility, Staphylococcus, hand rub, NICU

P582: **The mechanisms of resistance to ciprofloxacin among Acinetobacter baumannii isolates, isolated from nosocomial infections at Tehran hospitals**

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Background and Aim: The emergence of fluoroquinolone resistance is now of particular concern, given that relatively few antimicrobial agents are effective against A. baumannii.

Methods: Phenotypic and genotypic characteristics of resistant to ciprofloxacin among 100 Acinetobacter baumannii isolates, isolated from nosocomial infections at different hospital of Tehran were evaluated by E-test and PCR-sequencing methods. All isolates were then type by REP-PCR fingerprinting to finding the clonal relationship between resistant isolates.

Results: Our results indicated that resistant to ciprofloxacin among A. baumannii isolates in Tehran are increasing with resistant rate of 87% and highly ciprofloxacin resistant isolates have a mutation of Serine 83 ?Leucine in gyr A ORDRs. We cannot detect Par C mutations and plasmid-mediated quinolone resistance A among ciprofloxacin resistant isolates. REP-type A (55%), B (24%) and C (11%) were the most common types among A. baumannii isolates.

Conclusion: It seems that mutation in gyrA is a main mechanism of resistant to ciprofloxacin among A. baumannii isolates and the genes parC and qnr are not secondary targets for quinolones in ciprofloxacin resistant A. baumannii mutants in Iran.

Keywords: Ciprofloxacin resistant, A. baumannii, gyr A QRDRS

P583: **Frequency and Antimicrobial susceptibility of microbial agents isolated from Urinary Tract Infection (UTI) of Males in Ilam, 2011-2012**

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Background and Aim: Urinary Tract Infection (UTI) as a common clinical infection lead to refer to clinical centers. UTI is a most ordinary infection among young women, Children and sexual active persons. E.coli, Staphylococcus saprophyticus and Proteus spp. known as a most frequent agents of UTI. Raising of antimicrobial resistance among these agents cause occurrence of Urinary Tract Infection that shown resistance to broad spectrum of antibiotics. Current study is aimed to investigate the frequency and antimicrobial susceptibility of microbial agents isolated from UTI of Males during of 2011-2012 in Ilam

Methods: Data collected from hospitals and Clinical laboratories around Ilam through 2011-2012. Frequency and antimicrobial susceptibility of isolated agents investigated and compared with other similar studies in Iran.

Results: More than two third (64.8%) of the cases were female. E. coli (44.5%), Klebsiella (18.6%), Enterobacter (15 %) and Staphylococcus spp. (12.7%) were the most common microorganisms isolated from UTIs, respectively. High rates of resistance to tetracycline, ampicillin, and nalidixice acid were observed among these isolates.

Conclusion: Similar to other studies, E.coli was the most common bacteria causing UTI and showed a high rate of resistance against most of the antimicrobial agents. The use of appropriate antibiotics against UTIs and establishment of annual surveillance programs on determining the antimicrobial sensitivity of bacteria to routinely used antibiotics can be helpful for physicians in choosing a proper treatment in patients suffering from UTI and also to reduce the complications related to serious UTI

Keywords: Urinary Tract Infection, Drug resistance, infant and neonates, Iran

P584: **Comparison of Frequency and Antimicrobial susceptibility of microbial agents isolated from Urinary Tract Infection (UTI) of elder females by adult females in Ilam, 2011-2012**

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1- Dep. of Medical Microbiology, Ilam University of Medical sciences

Background and Aim: Urinary Tract Infection (UTI) as a common clinical infection lead to refer to clinical centers. UTI is a most ordinary infection among young women, Children and sexual active persons. E.coli, Staphylococcus saprophyticus and Proteus spp. known as a most frequent agents of UTI. Raising of antimicrobial resistance among these agents cause occurrence of Urinary Tract Infection that shown resistance to broad spectrum of antibiotics. Current study is aimed to investigate the frequency and antimicrobial susceptibility of microbial agents isolated from UTI of Males during of 2011-2012 in Ilam

Methods: Data collected from hospitals and Clinical laboratories around Ilam through 2011-2012. Frequency and antimicrobial susceptibility of isolated agents investigated and compared with other similar studies in Iran

Results: Among 395 females samples, 194 ones belonged to women whom were upper than 60 years and 201 of them were between 15 till 60 years. Most frequent UTI agents were Staphylococcus aureus (41%), E.coli (25%), Staphylococcus saprophyticus (20%) for upper 60 females. While 41% of UTIs in 15-60 ages females were E.coli and Staphylococcus aureus and Staphylococcus saprophyticus were 33% and 19%, respectively. Upper 60 females UTI antimicrobial resistance pattern were; Cefoxitin: 50% for S. aureus, Novobiocin: 83%, Tetracycline: 84% for E.coli. Cefoxitin: 60% for S. saprophyticus. UTIs antimicrobial resistance pattern for 15-60 ages were Amoxi Clav: 100% for E.coli, Cefamandole: 94% and Oxacilin: 89% for S. aureus Kanamycin: 100% and Oxacillin: 94% for S. saprophyticus.

Conclusion: These funding shown E.coli as first agent of UTIs in young females do not play main role among elder females but in both groups S. saprophyticus. Have similar frequency. Totally, antimicrobial resistance agents are most frequent among young females. These funding could be attributed to physiological change of females specially upper 60 ages. In other word, high frequency of antibiotic resistance illustrate there is not logical antibiotic prescription. Therefore, it is suggested there is need a policy to obligate antimicrobial susceptibility detection in advance of antibiotic prescription

Keywords: Urinary Tract Infection, female, Antimicrobial resistance, Ilam.

P585: **In vitro antifungal susceptibility testing of eight antifungal drugs against clinical and environmental isolates of Phaeoacremonium species**

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Background and Aim: Phaeoacremonium species are chiefly found in the environment and an uncommon cause of human disease. Most cases of Phaeoacremonium infection in humans involve traumatic inoculation and present as subcutaneous abscesses, cysts, or osteoarthritis. Of the many cases reported to date, a majority involve immunocompromised patients or immunocompromised. The ideal treatment for this infection is not defined, and the paucity of cases does not allow for meaningful comparisons of antifungal agents. In the present study, we have tested a total of 8 conventional and new antifungal drugs against clinical isolates clinical and environmental isolates of different species of Phaeoacremonium.

Methods: 66 clinical isolates from patients and 15 environmental isolates were taken from the reference collections of the CBS-KNAW Fungal Biodiversity Centre (CBS, Utrecht, Netherlands). All strains were identified to species level by sequencing of the β-tubulin genes. Microdilution testing was done in accordance with CLSI M38-A2 guidelines adjusted spectrophotometrically at 530 nm wavelength to optical densities that ranged from 0.17– 0.15 in RPMI 1640 MOPS broth with L-glutamine without bicarbonate. Plates were incubated at 35°C for 96 h. Quality control was performed by including Paecilomyces variotii (ATCC 22319), Candida parapsilosis (ATCC 22019), and C. krusei ATCC 6258.

Results: The resulting MIC90 s for all Phaeoacremonium species were in increasing order, as follows: posaconazole (0.125 ?g/ml); voriconazole (0.25 ?g/ml); amphotericin B (0.5 ?g/ml); isavuconazole (1 ?g/ml); caspofungin (8 ?g/ml); anidulafungin (4 ?g/ml); itraconazole (16 ?g/ml) and fluconazole (64 ?g/ml). Results shown that all Phaeoacremonium species are resistant to Itraconazole

Conclusion: Our results are in line with animal data, demonstrating that posaconazole and voriconazole had the highest in vitro antifungal activity against Phaeoacremonium species Isavuconazole seems to have also significant in vitro activity. Clinical effectiveness in the treatment of infection remains to be determined for these promising drugs

Keywords: Phaeoacremonium species, Antifungal activity, MIC90

P586: **Detection of the antiseptic-resistance genes in Pseudomonas and Acinetobacter spp. isolated from burn patients**

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Background and Aim: One of the major health problems in many parts of the world, is the treatment problems caused by burn injuries. Patients with such injuries are at increased risk of nosocomial infections. To avoid hospita-related infections, different kinds of antiseptic and disinfectant are used. Because opportunistic bacteria such as Pseudomonas aeruginos and Acinetobacter bumanii can locate on their burn injuries. Quaternary ammonium compounds (QAC) which contain benzalkonium cholaride as the most widely used agent, are employed as wound and skin antiseptics and as disinfectant in hospitals. The resistance mechanism to disinfectant occurs by genes which are resistance to quaternary ammonium compounds, namely, qacE type qacΔE1, qacΔE1 that are seperated from Gram-negative bacteria. Given the importance of prevention from nosocomical infections with origin of these two types of bacteria, the aim of this study was to determine the prevalence of antiseptic resistance genes qacE and qacΔE1 in clinical isolates Pseudomonas aeruginos and Acinetobacter bumanii.

Methods: In the study, 88 clinical isolate Pseudomonas aeruginos and Acinetobacter bumanii from burn hospital of Tehran province and Isfahan province in 1389-1390, were tested.To separate genes which are resistant against qacE, qacΔE1, PCR method was used.

Results: out of 83 clinical isolates of Pseudomonas aeruginos, 49 isolates (50%) had qacE gene and 76 isolates (91.5%) had qacΔE1 gene, and out of 5 clinical isolates of Acinetobacter bumanii 2 isolates (40%) had qac E gene and 4 isolates (80%) had qac ΔE1

Conclusion: This study showed that there are genes resistance to antiseptic in Pseudomonas aeruginos, Acinetobacter bumanii isolates, so that they make it difficult to prevent hospital infections and increase the risk of opportunistic bacteria for hospital patients specially those who have burn injuries.

Keywords: Pseudomonas aeruginos, Acinetobacter bumanii. Quaternary ammonium compounds (QAC), genes qacE, qacΔE1

P587: **Antimicrobial Susceptibility pattern of Klebsiella pneumoniae Strains Isolated From Educational Hospitals of Shahrekord**

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Background and Aim: The emergence of antibiotic resistance among pathogenic and commensal bacteria has become a serious problem worldwide. Klebsiellae are widespread in the environment and in the intestinal flora of humans and other mammals. Klebsiella pneumoniae is an important opportunistic pathogen causing urinary tract and intra-abdominal infections, neonatal meningitis and pneumonia in immunocompromised patients . The intensive use of broad-spectrum antibiotics in hospitalized patients led to the development of multidrug-resistant (MDR) strains. The worldwide development of MDR strains of K. pneumoniae is a matter of great concern.

Methods: 136 of Klebsiella pneumoniae clinical isolates were collected from educational hospitals of shahrekord and identified by using standard microbiological and biochemical tests. Antibiotic susceptibility pattern were determined by using disk diffusion method according to CLSI guidelines.

Results: Resistance of Klebsiella pneumoniae to various antibiotics was as follows: Amikacin (26.47%) ,cephalothin (53.67%) ,cotrimoxazole (61.02%) , Gentamicin(41.17%),cefotaxime(47.79%),nitrofurantoin(45.5%) nalidixicacid(33.08%) ,ciprofloxacin(21.32%). The most effective drugs against K. pneumoniae were imipenem (84.55%) and norfloxacin (76.47%) 93 isolates (68.33%) were detected to be MDR.

Conclusion: Multidrug-resistant Klebsiella pneumoniae is a major risk for hospitalized patients in shahrekord. It makes the treatment of infectious diseases difficult. so supervision on the consumption of antimicrobial agents and determining resistant strain can prevent development of resistance in bacteria.

Keywords: Klebsiella pneumoniae, Antibiotic resistance, multidrug resistant bacteria

P588: **Effect of Blood and Sera on growth of Staphylococcus aureus in BHI media**

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Background and Aim: Staphylococcus aureus growth rate is dependent to the component of culture media and addition of blood and sera are considered as stimulatory agant in the growth of many bacteria .The aim of this study was to assess the effect of either blood or sera in BHI media on the growth rate of S.aureus isolated from healthy carrier and clinical sources.

Methods: This study was carried out on five S.aureus isolates including two MRSA and two MSSA isolates which comprised one isolated from healthy carrier person and one from clinical sample, in each group and COL standard isolate. Plotting of growth curve and calculation of generation time of the isolates in BHI broth ,BHI containg sheep blood and BHI containing calf serum were carried out by inoculation 103cfuml-1 suspension of isolates in this three media . Then ,it was sampled and surface cultuered on MHA at hourly intervals through 24 hours.Colony count was carried out in every interval hourse quentially.

Results: The mean of generation time of five bacteria in three media of BHI, BHIS and BHIB was 25.38 ,26.2 and 26.9 min respectively which were not statistically significant (P>0.05). The generation time of standard strain was more than others.The mean generation time of MRSA was higher than MSSA isolates.This study showed that S.aureus which was isolated from healthy carriers had a slightly longer generation time than clinical isolates.

Conclusion: These results suggest that addition of sheep blood and calf sera in BHI media was not able to increase the growth rate of S.aureus, even it slightly increased the generation time. BHI is an enriched nutritious media and is ideal for growth S.aureus and addition of calf serum and sheep blood is not able to alter nutrition conditions in this media according to our assumption.

Keywords: Staphylococcus aureus,Growth curve,Generation time, MRSA,MSSA,Serum,Blood

P589: **Evaluation of antibacterial activity of Lemon verbena (Lippia citrodora) leaves**

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Background and Aim: Medicinal plants are the oldest known source for treatment of disease. Using pharmaceutical plants and plant extracts have been at great attention. In this research, the antibacterial activity of the ethanolic and aqueous extracts of Lemon verbena(Lippia citrodora) Leaves cultured in researchal farm Islamic Azad University , Azadshahr Branch were tested against nine bacteria strains including Bacillus cereus, Staphylococcus aureus, Pseudomonas aeroginosa, Salmonella typhimorium, Escherichia coli, Shigella dysentriae, Entrococcus fecalis, Yersinia entrocolitica and Staphylococcus epidermidis.

Methods: Crude extracts were obtained by using ethanol and hot sterile distilled water as the extraction solvent. Four concentrations (1000 mg/ml, 500 mg/ml, 250 mg/ml and 125 mg/ml) were used to check the antibacterial activity of this plant . The antibacterial activity of ethanolic and aqueous extracts was determined by by Disk and agar well diffusion methods.

Results: The most susceptible bacteria were to ethanolic extract were E. feacalis ,S. epidermidis , S. aureus and B. cereus while the most resistant bacteria were gram negative bacteria including Pseudomonas aeroginosa, Salmonella typhimorium and Shigella dysentriae. The largest zone of inhibition was against E. feacalis, S. epidermidis and S. aureus in agar well diffusion method 24, 22, 20 (mm) respectively, while in disk diffusion method largest zone of inhibition was against S. epidermidis and S. aureus 15 and 12mm respectively. Yersinia entrocolitica was the most susceptible among gram negative bacteria.

Conclusion: Effect of ethanolic extract of lemon verbena leaves was more than their aqueous extract and gram positive bacteria were more sensitive than gram negative bacteria.

Keywords: Antibacterial activity, Lemon verbena, Ethanolic and Aqueous extracts , Disk diffusion method, Agar well diffusion method

P590: **Prevalence of Acinetobacterbaumannii among hospitalized patients in Shiraz educational hospitals, Shiraz, Iran**

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Background and Aim: Acinetobacterbaumanniiis an opportunistic pathogen responsible for nosocomial infections, especially in intensive care units (ICU) and burn therapy units (BTU) patients.The majority of infections are of epidemic origin, and treatment has become difficult because many strains are resistant to a wide range of antibiotics. The aim of this work was to study the prevalence of A.baumanniiin Shiraz educational hospitals.

Methods: This cross-sectional study was performed in 3 hospitals during 6 months (December 2013 till May 2013). 2935 specimens(urine, wound, blood, sputum, endotracheal tube(ETT) and etc) were collected from patients referred to Shiraz educational hospitals (Faghihi, Aliasghar, Ghotebedin).These isolates were identified using standard biochemical and microbiological tests and then by Microgen® kit.

Results: Of 1027 culture positive urine samples,342 wound, 399 blood, 409 sputum and ETT samples and 713 positive culture of other clinical specimens,42 (3.9 %),, 69 (20.1 %), 28 (7.01 %),229 (55.9 %) and 13 (1.8 %) specimens were positive forA.baumannii respectively. 195 specimens (51.1 %) were from male and 186 (48.8 %) were female patients. all isolatedAcinetobacterbaumanniiin this study was resistant to all of the antibiotic tested including: Cefoxitin (SXT), Cefepim (CP), Solbactam (SAM), Imipenem (IMP), Ceftazidim (CAZ).24isolates (6.2 %) were susceptible to Gentamicin (3.1 %), Amikacin (3.1 %),and all isolates were susceptible to polymxin B.

Conclusion: The results of this study showed the prevalence of A.baumanniiis increasing in Shiraz hospitals. As increasing incidence of A.baumannii worldwide is a serious concern, so the control of this pathogenand considering preventive strategies is emphasized.

Keywords: Acinetobacter baumannii , Prevalence , Educational hospitals , Shiraz, Iran

P591: **Assessment of the effect of biosurfactant produced by Pseudomonas aeruginosa in lethal of Bacillus thuringiensis B. against white cabbage butterfly (Pieris brassicae L.)**

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Background and Aim: Surfactant production of cheap sources like oil sludge by biological agents such as bacteria, can be used in various industries. For example, in environmental processes such as bioremediation and elimination of environmental pollutants and as a distributor pesticides in agriculture as well as synergistic agents can be used.

Methods: In this study, produced surfactant by Pseudomonas aeruginosa (isolated from petroleum sludge) at different times (24, 48, 72 and 96 hours) with chemical surfactant Tween- 80 and the biological control agent, Bacillus thuringiensis, were used in a pilot project on the cabbage pests (Pieris brassicae) and their synergistic properties were evaluated.

Results: Statistical analysis of the results of Bacillus thuringiensis treatments with surfactant produced at different times, chemical surfactant Tween-80 and control treatment showed significant differences($P=0.05$). This research showed that surfactant treatment produced in 24 and 48 h, has the greatest synergistic effect and is in an equal group with chemical surfactant treatment.

Conclusion: From the study it is concluded that in addition to the surfactants biological roles in bioremediation, it can be used as synergistic agent against plant pests and be a viable alternative to non-economical chemical surfactants that annually enters millions of tons of harmful chemicals substances into the fields and underground water.

Keywords: Bacillus thuringiensis, Surfactant, Tween-80, pesticides, Pieris brassicae, synergistic agents.

P592: **molecular study of native isolates of bacillus thuringiensis and their effect on Drosophilla melanogaster**

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Background and Aim: Bacillus thuringiensis is a known bacterium for biological control of pests. This bacterium is gram-positive, spore-producing and saprophyte. Its main feature is the production of crystalline body in stagnation phase that have specific killing for different orders of insect. This crystal body containing two groups of toxins involving Cry and Cyt toxins. Cry toxins have different types that each of them have specific toxic effect for different orders of insects such as Lepidoptera, Coleoptera, Diptera and others and for other animals are ineffective. However Cyt toxins only have been seen in Dipterans insects. Effective isolates with Cry toxins has synergetic effect with effective Cry toxin. The dipteran order is including insects that some of them are vectors of human pathogen and also some are important agricultural pests. Studies have been shown that isolates of this bacterium that is effective against agricultural pests are very rare.

Methods: Therefore in this study nineteen isolates that were isolated from soils of Isfahan, Tehran and Mazandaran regions and six isolates that were provided by Seed and Plant Improvement Institute, that previously was isolated from Mashhad were used. PCR reaction for Cry4A, Cry4B, Cry10, Cry11, Cyt1 and Cyt2 genes, that is specific for dipteran insects were applied. Furthermore bioassay reaction against fruit fly, Drosophilla melanogaster was performed.

Results: The result showed that Cry4A, Cry4B, Cry10 and Cry11 genes have 24, 44, 32, 32, 8 and 20 percent frequency, respectively. Observation of bioassay reaction show that majority of isolates don't show lethality and a few number have toxicity against this insect. Among the isolates, one of them, isolate 402, has six detected genes and the highest mortality.

Conclusion: Therefore it can be concluded that isolate 402 is more effective among 25 isolates and other tested and should be applied in biological control programs of dipterans insects.

Keywords: Bacillus thuringiensis , Cry toxins, Drosophilla melanogaster, PCR

P593: **Study of ability of native Bacillus thuringiensis isolates for human blood cells hemolysis**

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Background and Aim: Bacillus thuringiensis is a known bacterium for biological control of agricultural pests. It is Gram-positive, spore-producing aerobic and saprophyte. This bacterium has two phases of growth that are vegetative and stagnation phases. In any phase, the bacterium will produce different toxins. For example, toxins VIP, beta-exotoxins and hemolysin in the vegetative phase and production of Cry and Cyt toxins are in stagnation phases. The identification of presence of toxins in isolates and their performance is important for selection of efficient isolates on pest also safe for environment and invertebrates.

Methods: This study evaluates the ability of native B. thuringiensis isolates for human blood hemolysis test. First, the ability of each isolate to hemolysis and secondly to determine the prevalence of bacterial strains that are able to hemolyse human blood cells. In this study native B. thuringiensis isolates were cultured on blood agar medium plates and incubated for 48 h at 37 °C and have been tested for human blood hemolysis reaction.

Results: . Results showed that fifty-six percent of the isolates aren't capable of human blood hemolysis, beta hemolysis was forty percent and four percent showed alpha hemolysis.

Conclusion: . In the case of application of this isolates for biologic control of pest, hemolysis reaction should be observed that have been used for safe isolates to human.

Keywords:: Bacillus thuringiensis, blood hemolysis, native isolate

P594: **Isolation and identification of gamma radiation resistant bacteria from Lout desert of Iran**

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Background and Aim: The bacteria that live in dry and arid places have special repair mechanisms. These bacteria are resistant to ionizing radiation as well as desiccation, and are able to repair genome damages. The aim of this research was isolation, identification and survey of gamma radiation resistant bacteria from the soil of arid places in lout desert.

Methods: Soil samples were collected from different areas of lout desert. To remove other microorganisms from soil, primary treatment with 10 kGy of gamma radiation was performed then transferred into TSB medium. In order to find resistance of bacteria to gamma radiation, strains were exposed to different doses of gamma radiation and survival of bacteria was calculated. Phylogenetic characterization was undertaken using molecular analysis of 16s rRNA gene.

Results: From 20 isolates, 3 strains LD4, LD5 and LD7 were shown more resistance to gamma radiation. These strains were gram negative, rod shape and had pink colored colonies. Strain LD4 was resistant to 15 kGy of gamma radiation while LD5 and LD7 were resistant to 10 kGy.

Conclusion: According to high resistance against gamma radiation, these isolates are proposed to have the potential for removing heavy metals and also bioremediation of radiation polluted sewage.

Keywords: Gamma radiation, Resistant bacteria, Lout desert

P595: **Isolation of Nodule-Associated Pseudomonas sp. From the Root Nodules of Alfalfa andTheir Possible Role in Plant Growth Promotion**

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Background and Aim: Alfalfa plant.

Methods: 16srRNA.

Results: times.

Conclusion: Alfalfa plants.

Keywords: , Nodul, alfalfa

P596: **Evaluation of different media for isolation of Rhodococcus from agricultural lands and identification of the isolates using API Coryne system**

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Background and Aim: Rhodococci are widely distributed in nature and have been frequently isolated from soil, fresh water, gut of some insects and so on. This bacterium may play an important role in the biotransformation of chlorinated phenols in the environment. Therefore, the aim of this study was isolation and characterization of Rhodococcus from soil using APi Coryne kit and evaluation of best medium for isolation.

Methods: Totally 120 soil samples have been collected from agricultural lands in Qom city. The samples were serially dilluted (10 -1- 10-7) and cultivated on different media including: ISP5, BHI and Bennet Agar media. The C for 48 hrs and then the pure isolates were°plates were incubated at 30 rieux).′identified using APi Coryne kits (bioMe

Results: In general, 265 bacterial strains were isolated. Out of that 52 strains were gram positive, strict aerobic, partially acid fast, and non – endospore forming bacteria. Among the isolated bacteria two isolates belong to Rhodococcus and identified as Rhodococcus equi and Rhodococcus erythropolis. In addition the results showed that the best medium for isolation was ISP5 agar.

Conclusion: Overall, the results obtained from this study indicated that contamination of soil by pesticides and pollutants can provide the best condition for screening of Rhodococcus bacteria

Keywords: Rhodococcus, APi Coryne system, ISP5 medium

P597: **Isolation And Identification Of Lactic Acid Bacteria Found In The Honey Stomach Of The Worker Honeybee From Tonekabon Area And Survey Antibiotic Resistance**

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Background and Aim: Lactic acid bacteria (LAB) form a taxonomically diverse group of microorganisms that can convert fermentable carbohydrates into lactic acids. Although the use of LAB has a long and safe history and has acquired the 'generally regarded as safe' (GRAS) status, the safety of selected strains should be evaluated before use. Enterococci are members of the lactic acid bacteria (LAB) group which consist of Gram-positive, catalase-negative cocci or rods that produce mainly lactic acid from the fermentation of carbohydrates. The honey stomach, when filled with nectar and nutrients, has a microaerophilic state and is at an optimal temperature of 35°C in the hive, and thus, it represents an optimal niche for the LAB. Hence latter study concentrates on isolation, analysis probiotic properties and survey antibiotic resistance lactic acid bacteria found in the honey stomach from the worker honeybee.

Methods: LAB were isolated from the honey stomach on MRS, M17agar medium under anaerobic conditions using anaerobic jars with Anaerocult C gas packs and at 37°C. Using an initial screening such as, Gram-positive, catalase-negative and SIM, cocci were chosen. Then the 16S rRNA genes from extracted DNA of bacterial colonies were amplified with polymerase chain reaction using universal primers (8F and 1492R) and were sequenced.pH and bile salt tolerance tests were used to determine the probiotic potential. Disc diffusion method was used to screen for the antibiotic resistance of isolates with 11 discs containing Ampicillin (AM 10 µg), Trimethoprim/sulfamethoxazol (SXT 25µg), Cephotaxim(CTX 30 µg), Vancomycin (VA 30µg), Piperacillin(PIP 100µg), Ticarcilin(TIC 75µg), Tetracycline (TE 30µg), Penicillin(P 10µg), Gentamicin (GM 10µg), Doxycycline (D 30µg), Ciprofloxacin (CIP 5µg) and Inhibition-zone diameters were measured after anaerobic incubation at 37˚c for 24 h.

Results: Total from the MRS and M17 plates, 33 different colonies were picked up for the biochemical tests, and of these, 12 plates were cocci gram-positive, catalase-negative all of which were non-motile. Randomly, three cocci were subjected to sequence analysis that 2 isolated strains (M1A9, K1.S8) showed a similarity 99% (Enterococcus durans strain M.D.E MRS4-10, Enterococcus faecium strain PON94) and 1 isolated strains(H1.S3) showed a similarity 100% (Enterococcus faecium strain HB2003) with database sequences in NCBI. 2 isolated strains (M1A9, K1.S8), strains were high tolerance against acidic conditions and bile salt, but isolated strain (H1.S3) was introduced bile salt sensitive strain. All isolates were resistant to Pinicillin, Piperacillin and Vancomycin. None of the isolates no growth in presence Tetracycline, Ampicillin and Doxycycline, in other words, isolates were sensitive to latter antibiotics.

Conclusion: Through exposure to honeybees with flowers, we can say that LAB found in the honey stomach associated and dependent ex parte with insect and on the other side with LAB found in flowers and plants.Enterococcus are LAB with important probiotic activity which are used in the food industry as starter cultures, also responsible for peculiar sensory characteristics of many fermented products. on the other, Enterococcus Spp can harbor pathogenic markers and be resistant to antibiotics of clinical relevance.hence,should be more carefully considered in use them as probiotic.

Keywords: lactic acid bacteria, honey stomach, worker honeybee, antibiotic resistance

P598: **Introduction of Efficient Novel Method for DNA Extraction from Soil**

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Background and Aim: Isolation of nucleic acids from natural environments is one of the basicsteps for the study of microbial communities, including cultured and uncultured microorganisms. Among nucleic acids, DNA has been of growing interest for the study of diversity and phylogenetic properties of microorganisms as well as for the construction of metagenomic libraries. Various methods are introduced to extract the environmental DNA (eDNA) from diverse compositions. But none of these methods are perfect and applicable to different type of soils. Here we introduced a high yield novel method for DNA extraction from various soil samples.

Methods: Loamy (L), sandy (S) and rhizospheric soil of (R) were used for DNA extraction. One gram of the soil samples was weighted and sieved with 1mm mesh and pounded with mortar and pestle. Then, 5ml lysis buffer(Z buffer, 100mM Tris-HCl, 100mMNaPhosphate solution, 100mM EDTA, 1.5M NaCl, H2O pH: 8.0) was added to the sample. Three methods of DNA extraction were studied: A method: Liquid nitrogen incursion was performed. Lysis of microbial cells was donebylysozyme solution .Then, glass beads were added and the mixture was shaked for 1 hour in 200rpm. B method: Glass beads were added to the sample and vortexed. Lysozyme was used as describe in Amethod. In the last lysis step, 3 cycles of freeze-thaw(-70oC and 65oC, respectively) were done. C method: After incursion in liquid nitrogen, the samples were sonicated at 37 Hz for 20 minutes. Then, freeze-thaw cycles were performed as described in B method. In all of methods, the samples were treated by 20% SDS at 65oC. The samples were centrifuged and the supernatant was used for DNA extraction using Phenol: chloroform extraction method.The pellet (DNA) was resuspended in T10E10 buffer(1M Tris-HCl, 0.5M EDTA, H2O, pH: 8.0). Gel electrophoresis method was used to detect the DNA.

Results: The quantity and quality of extracted DNA were differed in the three methods. Minimum and maximum amount of DNA was achieved in the C and A methods, respectively. Amount of extracted DNA by A method was 3 and 6 times higher than that of B and C method, respectively. Concentration of DNA in R soil is higher than that of S and L soils.

Conclusion: In the presented study a novel method for DNA extraction from soil samples is introduced. The cells of in the soil are efficiently disrupted by incursion in liquid nitrogen, bead beating and lysozyme.This method has suitable application on extraction of DNA from loam, sandy and rhizospheric soils. Higher amount of DNA extracted from the R soil is may be due to more microbial richness of this type of soil than that of other soil types studied. This method is an efficient and useful method for environmental metagenomics studies.

Keywords: DNA, Metagenomics, Rhizosphere, Soil

P599: **Isolation of heavy metal resistant siderophore producer's actinomycetes from soils**

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Background and Aim: Heavy metals have been expanding in ecosystems by biochemical reactions and human activities. Microorganisms employ several mechanisms to resistance to heavy metals .One strategy is the immobilization of metals to reduce entrance to the cells. Production of siderophores are an efficient way to chelate metals outside of the cell. Siderophores have low molecular weight with tendency to bind to the iron and facilitate formation of soluble form of iron. Siderophores can bind to other metals, such as cadmium and zinc, too. The aim of this study was isolation of metal resistant actinomycetes with siderophore production capability.

Methods: The soil samples were collected from pollutant areas. Primary screening of metal resistant actinomycetes was performed on soil extract agar and tap water agar supplemented with heavy metal salt solutions (cadmium chloride, nickel chloride, copper sulphate, lead acetate, zinc chloride). The resistant isolates were selected for determination of minimum inhibitory concentrations of metals. The selected resistant actinomycetes were screened for presence of siderophoric compounds. The resistant isolates were inoculated to the chemically defined medium supplemented with metal salt solutions. After one week cells were harvested via centrifugation and the supernatants were collected for siderophore assay using chromo azuerol sulphate (CAS)as an indicator.

Results: From 30 soil samples, 40 metal resistant strains were isolated. Among 14 metal resistant isolates, 8 strains were able to produce siderophore agents. The strains UTMC 2179, UTMC 2200 and UTMC 2190 showed high resistance to Zn+2(140 mM). Strains UTMC 2241 and UTMC 2242 were able to grow on 9.2 mM and 6 mM Cd+2, respectively. UTMC 2236 was resistant up to 70 mM Zn+2. MoreoverUTMC 2241and UTMC 2200 showed resistance to 7 mM Cu+2. It is noticeable that in fermentation broths of UTMC 2163 and UTMC 2195, color of CAS changed immediately after addition of the indicator. They were also resistant to 6.9 and 4.6 mM cadmium, respectively. Both of them were able to grow on 3.5 mM Cu+2, too. Molecular analysis indicated that UTMC 2163 belonged to genusSaccharothrix and the rest of strains recognized as Streptomycessp.strains.

Conclusion: In this study we isolated metal resistant actinomycetes which were able to produce siderophore as a way to resist toward a high concentration of heavy metals. UTMC 2241 and UTMC 2200 due to their resistance to high levels of metals and resistance to multiplex metals were selected for further studies on involvement of scavenging metabolites in their extreme metal resistance trait.

Keywords: Actinomycetes, Heavy metal, Siderophore, Metal resistance

P600: **Quantitative assay of Bacteriorhodopsinin in isolated Bacterioruberin mutants of Halobacterium salinarumR1**

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Background and Aim: Bacteriorhodopsin(BOP),a trans membrane proton pomp, is a well characterized membrane protein. Bacterioruberin is the main carotenoid of the H. salinarum that make problems in the BOP purification. Isolation of bacterioruberin deficient and bacterio-opsin producing mutants of H. salinarum R1 was the main purpose of this research.

Methods: Bacteria and Culture: HalobacteriumSalinarum R1was obtained from the DSMZ Germany. The liquid growth medium contained 250 g/L NaCl, 20 g/L MgSo4.7H2O, 2 g/L KCL, 3 g/L Na3-citrate, 3 g/L yeast, 5 g/l casein and solid medium had additional 15 g/l agar. The media adjusted to pH 7 to 7.2. UV radiation: Cells collected in exponential phase using centrifugation at 4000 rpm for 10 minutes and resuspended in fresh medium to give 107 cells per ml. 1 milliliter of suspension spread over surface of a plate and irradiated by 8 Watt short-wave UV lamp for 25 minutes. Selection of mutants based on lack of red color. Analysis of bacterioruberin absorption spectrum: 2.5 ml of cell suspension of each mutant in stationary phase of growth, collected by centrifugation at 10,000 rpm for 5 minutes. Supernatant discarded and to 1 milliliter of hot ethyl alcohol added the pellets and vigorously mixed. Then, the samples were centrifuged at 1000 rpm for 2 minutes and absorption of each supernatant determined using spectrophotometer. Quantifying of bacteriorhodopsin: 2 milliliter of bacterial suspension in stationary phase centrifuged at 12,000 rpm for 5 minutes. Then 1 milliliter deionized double distilled water containing 0.01 milligram DNase I added to the pellets. Samples mixed vigorously. Afterwards, they were mixed in darkness with NaoH 4M and NH2OH 4M in the proportion of 9: 0.5: 0.5. Finally, the level of bacteriorhodopsin in samples figured out using Oesterhelt,and colleagues' (1974) equation.

Results: Results: in this research 11 mutants were detected and studied. The level of bacterioruberin in samples were significantly lower than that of controls; mutants number 2 and 6 bear minimum level of bacterioruberin. Also results of bacteriorhodopsin quantification illustrated the mutants that depicted by number 2,5,6 and 28 were same as non-mutated H. salinarumR1.

Conclusion: Discussion: one of the first defense mechanisms of Halobacterium salinarum against environmental harsh conditions is the presence of carotenoid pigments such as bacterioruberin; in wild-type bacterium the presence of high amounts of this membranous pigment guards cells against UV-related damages that as a contaminant interfere with purification of bacteriorhodopsin from this organism; this carotenoid is lost in some of isolated studied mutants. Although bacterioruberin production pathways In selected mutants (number 2 and 6) is damaged to a certain extent, bacteriorhodopsin expression pathways is still active.

Keywords: Halobacterium salinarum ,bacterioruberin,bacteriorhodopsin

P601: **Evaluation of Halobacterium salinarum R1resistance against UV radiation**

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Background and Aim: Halinobacterium salinarum is an archaebacterium that commonly live in the extremely salty environments. The purpose of this research was determination of resistance of Halinobacterium salinarum against ultraviolet radiation.

Methods: Culture medium consisted of 250 g/LNaCl, 20 g/L MgSo4.7H2O, 2 g/L KCL, 3 g/L Na3-citrate, 3 g/L yeast, 5 g/l casein and solid medium had 15 g/l agar. The media adjusted to pH 7 to 7.2) UV radiation: One milliliter of exponential phase of microbial suspension with 107 cells per ml spread over the surface of sterile plate. The plate was irradiated with an 8 Watt short-wave UV lamp at 30 cm distance in absence of visible lightwith 0.4 joule per square meter intensity. To prevent activation of repair processes the plates covered by aluminum foil. After 9 days incubation at 37oC, mutated colonies with altered color counted.Survival curve was plotted based on the number of appeared colonies each radiation intervals and incubation of irradiated cells.In order to examine the effect of photo-reactivation process on the bacterial survival, after 20 minutes of UV radiation, we cultured exposed microorganisms in the presence and absence of visible light.

Results: mutation rate was calculated based on the numbers of mutated colonies divided by total number of colonies, which was determined as 0.00026. In this research 11 mutant colonies isolated that stayed alive after several subcultures. Survival curve revealed,although the rate of death in this microorganism was notably associated withthe increase in the UV radiation, itsurvived after 30 minutes UV exposure ; the number of total living cells in the absence of radiation and after 30 minutes of radiation, was 3x1010 and 5x108, respectively. Growth in the presence of visible light results in high cellular density (OD) in comparison absence of light that is in consistent with high impact of photo-reactivation on cellular repair system.

Conclusion: According to the results of this study, both high resistance to UV radiation and presumably the presence of light inducedrepair mechanisms in this archaebacterium participate in the durability of it under the harsh environmental conditions.

Keywords: Halinobacterium salinarum, UV,repair

P602: **Isolation of Bacillus megaterium 37-1 Strain from Soils Treated with Dairy Waste**

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Background and Aim: Lipase has an important place among biocatalysts. Lipase enzyme that produced by Alkalophilic Bacteria in industry has a great importance. The aim of this study, isolation and molecular identification of Bacillus lipase-producing from soil of Mazandaran province west areas.

Methods: 50 soil samples from a depth of 15-10 cm in rural areas were taken. After thermal treatment and preparation serial dilutions, was cultured on Tributyrin Agar media with glycerol butyrate lipid substrate. The next step, Bacillus isolates were selected with a clear halo zone diameter at different temperatures was measured. The isolated Bacteria with maximum diameter of lipase activity were selected and their DNA was extracted. Identification through the PCR 16S rDNA, sequencing and aligning sequences was designated in gene bank. Lipase activity of isolated enzyme culture through the relative concentration of supernatant resulting from culture media in different temperatures and concentrations of substrate were prepared and determined with a spectrophotometric method. The relative molecular weight of the enzyme was determined by SDS-PAGE and comparison was determine by protein syndrome.

Results: After screening soil samples, 5 gram-positive Separated were isolation as Bacillus by morphological and biochemical characteristics. Isolated 1-37 with the largest halo zone diameter in mm 17 under 40 C° temperature, after sequencing and partial aligning sequences of 16S rDNA were recorded 37-1 Bacillus megaterium strain in gene bank. The relative molecular weight of lipase strain, was set equal KDa 55. The maximum lipase activity respectively amount 0/626 U/ml at 55 C° in pH 9. Km and Vmax Parameters was infectious with using Lynvrbrg and michaelis-menten curve.

Conclusion: The results of this study revealed that the lipase-producing bacterial strains isolated from soils treated with dairy waste was Bacillus megaterium 37-1 strains. In this study, the Vmax parameter at 37 C° has a greater amount of 55 C° equal to 6/527 and Km index in this temperature was measured amount of 3/471, which represents the efficiency of the Strain lipase enzyme in 37 C° is more.

Keywords: Bacillus megaterium, Soil, Dairy waste.

P603: **The effect of carboxin-thiram fungicide on viability and effectiveness of some selected plant growth promoting Azospirillum strains in wheat seed**

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Background and Aim: In addition to that biofertilizers have positive effects on soil stable fertility; they also have some other beneficial attributes such as economical and environmental. Therefore they can be used as a suitable substitute to replace all or at least some of chemical fertilizers. Biofertilizers include Plant Growth Promoting Rhizobacteria (PGPR). One of the most important components of PGPR group is Azosprillum. These bacteria can increase plant growth through direct and indirect mechanisms. Considering wide use of Carboxin thiram to control fungus pathogens in wheat cultivation in Iran, this project was designed and performed to define the effects of this fungicide on the establishing association relation with species of Azosprillum.

Methods: Performing green house test, use and non-use Carboxin thiram effects on growth indexes of wheat plant (Chamran Cultivar) association conditions with 5 Azosprillum species (A.brasilense, A.lipoferum, A.halopraeferense, A.irakense, A.sp) were studied. Also effect of Carboxin thiram on these 5 Azosprillum species inoculant formulations (powder and liquid) was surveyed in vitro condition.

Results: Bacteria counting test results showed that bacteria number on seed in powder formulation in Carboxin thiram use level was 6.5×108 and in non-use Carboxin thiram level It was 1.9×108. It demonstrates that this fungicide has no negative effect on seed stability in this formulation. This value is decreased one logarithmic value in liquid formulation with using Carboxin thiram (4.7×107). Final results showed that in bacteria and Carboxin thiram interaction, tillering, node interface, flag length, seed number, seed weight, plant wet matter weight ($p<0.01$), and also 1000 seed weight and plant dry matter weight ($p<0.05$) were significant.

Conclusion: Finally we can say that not in all but in most of the measured indexes, A.irakense and A.lipoferum had better situation rather than another treatments in Carboxin thiram presence.

Keywords: Azosperillum, Carboxin thiram anti fungi, Plant growth promoting bacteria, wheat.

P604: **Screening and isolation of high heavy metal TolerantBacteria (CPPb-62)fromcontaminated soils**

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Background and Aim: Heavy metal is a toxic produced as a by-product of several industrial processes. Thus, strategies employed to decontaminate environmental toxic metal focus on removing it. Microorganisms such as bacteria have developed the capabilities to tolerant heavy metal toxicity by various mechanisms. Many microorganisms have been reported to reduce the highly toxic Cr6+, Hg2+, Cd2+ , Pb2+ and Co2+ to the less toxic. The present work deals with the isolation of resistant bacteria from contaminated soil, its molecular characterization, and evaluates the mechanism of these bacteria to detoxification of various heavy metals.

Methods: Growth curves of isolated strains were determined in LB medium supplemented with the desired toxic metal concentration. The Minimum Inhibitory Concentration was also determined for the same strains. Heavy metal in the culture supernatant was measured using desired methods.

Results: We investigated the microbial resistant/tolerance in contaminated soils. The isolated strains were resistant to Cr6+, Co2+ and Pb2+ upto 500ppm,1500ppm ,30000ppm respectively. For more studied of mechanism of detoxification heavy metal, Atomic Force Microscope image and FTIR were obtained. Result showed that in the case of Cr6+ the concentration of this toxic metal in medium after 24h decreased to 50%.

Conclusion: This strain was highly tolerant in the wide range of carcinogenic heavy metals and removes these metals with various biological mechanisms.

Keywords: Heavy metal resistance bacteria, Bioremediation, Atomic force microscope

P605: **CPPb-92: a heavy metal resistancebacterial strain; Identification and evaluation of its potential for bioremediation**

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Background and Aim: Heavy metal wastewater exists in various industrieswhich threaten to the environment andhuman lives severely.Although heavy metal removal from aqueous solutionscan be achieved by conventional methods, but they may beineffective or cost-expensive.Microbe-based removal is now considered to be an effective alternative method to the conventional processes and is receiving greater levels of interest for potential uses in bioremediation. Generally, microorganisms have evolved various measures to respond to heavy metal stress via processes such as transport across the cell membrane, biosorption to cell walls, entrapment in extracellular capsules,as well as enzymatic transformation to other, less toxic chemical species by redox reactions. Their detoxifying mechanismscan be applied to design economical bioremediationprocesses.The aim of this study was toidentification of heavy metal-resistance bacterial isolates couldpotentially use in bioremediation of noxious chemicals contaminated environment.

Methods: The minimal inhibitory concentration (MIC) of heavy metals atwhich no colony growth occurred was determined in triplicate inLB liquid medium with a series concentration of Cr6+, Hg2+, Cd2+ , Pb2+ and Co2+.FurthermoreGrowth was examined in presence of desired concentration of this metals. Resistance mechanism in this strain had evaluated by spectroscopic techniquessuch as FTIR.

Results: This strain displayed high tolerance capacity with MIC values of 5000ppm,70000ppm toCr6+ and Pb2+ respectively. Results had approved heavy metal uptake by this strain and diminution in remained concentration of metal in culture supernatant. In the case of Chromium this reduction was 50% after10h.Adsorption of metals in cell surface of this strain was confirmed.

Conclusion: The carcinogenic heavy metal bioreduction/bioabsorbtioncapacity of isolated strain was benefit for biotechnological applications and bioremediation.

Keywords: Bioremediation,Fourier transform infrared spectroscopy, Heavy metal uptake

P606: **Isolation and growth of Magnetotactic bacteria from Mighan peatland and Iron Ore in Iran.**

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Background and Aim: Magnetotactic bacteria reacts to the Earth's magnetic field due to their ultra-structure of the bacterial magnetosome are unique. In this study, isolation Magnetotactic bacteria of different climates have been made and characterization of their nanoparticles. These two regions were Peatland Mighan and Iron ore.

Methods: Sampling was conducted of areas such as Peatlands Mighan,Morvarid iron ore. In order to establish the optimum conditions for oxygen and magnetic field of the anaerobically state and in the presence of 1.46 Tesla magnet specific bacteria in a culture medium, were stored for one month. DNA extracted, analyzed by transmission electron microscopy and strain mapping in the end.

Results: A total of 15 samples from different points, selected the only 6 samples containing the sediment sample. When the bacteria in low oxygen conditions, were affected by the magnet in response moved into it. Transmission electron microscopy observations on the presence of the ultra-structure of intracellular magnetosome nanoparticles were synthesized proved. Gram staining and was confirmed gram negative bacteria. Revealed DNA extraction and PCR only Iron ore samples was then BLAST. Strains were related genus of Magnetospirillum magnetotacticum.

Conclusion: This study examined the microbiological aspects of magnetotatic bacteria and the presence of them found in Mighan wetlands, are not unique habitats of these bacteria such as iron ore is rich in iron ions.

Keywords: Magnetotactic bacteria, Magnetic Nanoparticles , Transmission electron microscopy, Magnetospirillum magnetotacticum

P607: **Detection of streptomyces species diversity by using ERIC-PCR technique**

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Background and Aim: Nowadays enzymes are being isolated from streptomyces that has many uses in industry. It is obvious that the differences between bacterium strains causes different antibiotics hence for the use of enzymes and metabolites of this bacterium in the world and introducing of a new sample of metabolites, researchers are permanently studying the bacterial diversity of soil types in order to introduce a new strain of the bacterium with the ability of different antibiotics and other enzymes. Country of Iran because of his four seasons climate and the high extent of ecosystem and climate ,It may has a new type of bacteria.

Methods: In this study in order to identify and evaluate diversity of Streptomyces, soil samples from different areas of Ardebil, Astara, Meshkin, Sarein in different seasons was gathered. By Transferring soil samples to laboratory soil pH was measured and after dilution in medium, specific SCA were cultured.

Results: Then After re-culturing in liquid medium ISP, DNA extraction was performed and by using gene specific primers, 16SrDNA were reproduced.

Conclusion: . After identifying bacterial species by using ERIC-PCR technique, the species diversity of bacteria isolating were investigated and by using the software NTSYS their pedigrees were drawn.

Keywords: Streptomyces, Genetic variation, Ardabil, ERIC-PCR

P608: **Study on application multiplex PCR for detection of salmonella typhi and E.coli O157: H7 in the Karoon river**

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Background and Aim: Water is a vital element withstriking and uniquefeatures.Which is one of the most important chemical elements that makes huge amount of environment. Salmonella typhi& E.coliO157are Pathogenicmicroorganisms that can cause severe Gastrovantrit in human being. These Pathogens can be transferred in different ways such as water and food.existence of E.coli andSalmonellain surface water are threat to human'shealth. Identifyingand counting water source Pathogens with common bio chemical faces with problems and limitations.

Methods: PCR (Polymerase Chain Reaction) is one of the molecular methods that can replace current experimentation. Recent way is susceptible, exact, cheap and fast. With this method even on bacteria in 100 ML can be identified. In this research we observe indicator bacterialspecies of Pathogens in Karoonriver in Ahvaz realm. Hence multiplex PCR technic is used due to its simultaneousdetectioncapability of microorganism.Rfb primer is used to diagnoseE.coli and InvA primer for Salmonella typhi.

Results: Results of observing 50 samples indicate to exist of Salmonella typh&E.coliO157 in Karoonriver in Ahvaz realm. And these bacteria are deleted by Chlorine in disinfection andtreatment levels.

Conclusion: these bacteria are deleted by Chlorine in disinfection and treatment levels.

Keywords: Mutiplex PCR ,Salmonella typhi , E.coli

P609: **Bacterial contamination in the surfaces of different wards from a tertiary hospital at central Iran**

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Background and Aim: Hospital environment play an important role in nosocomial infection, as the health-care environment contains a diverse population of microorganisms. In this setting, microbiologically contaminated surfaces can serve as reservoirs of potential pathogens. An understanding of how infection occurs after exposure is based on the principles of the chain of infection, which is also important in evaluating the contribution of the environment to health-care-associated diseases In our hospital (Vali-Asr, Arak, Iran), the rate of nosocomial infection with microorganism like S.aureus, Acinetobacter and enterobacteriacae is increasing. The aim of present study is to investigate the presence of common microorganisms present on hospital surfaces in different wards.

Methods: A total of 190 baseline swabs were taken from different wards in valiasr_hospital. The samples were randomly collected using moistened swabs in normal saline from high and low hospital contact surfaces.

Results: Out of 190 swabs, 58 were negative and from 132 culture positive samples, 203 isolates were identified. Among these bacteria the frequency of Staphylococcus aureus was 7(3.5%), methicillin resistance Staphylococcus aureus 6 (3.5%), Staphylococcus saprophyticus 44(22%), Staphylococcus epidermidis 49(22%), staphylococcus haemolyticus 78(39%), klebsiella pneumoniae 9(4.5%), Enterobacter aerogenes 4(2%), acinetobacter baumanii 4(2%) and alcaligenes2(1%) isolated.

Conclusion: According to the results of present study the prevalence of pathogenic microorganism in the surface of this hospital setting is considerable and these environments might act as environmental vehicles for the transmission of potentially pathogenic bacteria. Moreover, methods of decontamination and disinfection of the surface must be revised and further research should focus on the efficacy of the disinfectant agents.

Keywords: nosocomial infection, hospital environment , pathogenic bacteria

P610: **Antibacterial effects of oral drop of lemon, Saliva officinalis and methanol extract of Medicago sativa**

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Background and Aim: Due to the side effect of chemical drugs and increasing antibiotic resistance, determination of antibacterial activity of plants and natural products

Methods: the expert. Shade dried aerial parts of the alfalfa plants were used for preparation of extract by percolation method using 100% methanol. Impregnated discs containing 4 mg of alfalfa extract and 75 µl of oral drop of lemon and S. officinalis (Pars Ataran- Iran) were prepared. The antibacterial activity of extracts was evaluated by Disc Diffusion method according to the standard Kirby-Bauer against 10 clinical isolates and one standard strain of each bacterium. Gentamicin disks (30 µg) were used as positive controls. Results: The mean diameter of lemon inhibition zone against E. coli, P. aeruginosa and S. epidermidis was respectively 7.4, 8 and 9.6 mm. S. officinalis had no effect against any of the tested bacteria. Alfalfa extract had no effect against P. aeruginosa. The minimum inhibitory concentration for oral drop of lemon was 1/2

Results: The mean diameter of lemon inhibition zone against E. coli, P. aeruginosa and S. epidermidis was respectively 7.4, 8 and 9.6 mm. S. officinalis had no effect against any of the tested bacteria. Alfalfa extract had no effect against P. aeruginosa. The minimum inhibitory concentration for oral drop of lemon was 1/2

Conclusion: Herbal compounds have been used in the prevention and treatment of disease for a long time. The results of this study showed that oral drop of lemon was more effective against tested bacteria and is suggested the evaluation of antimicrobial effects of its active components in future research activities

Keywords: Antibacterial effects.oral drop of lemon.Saliva officinalis

P611: **Fe (III) Reduction by Halomonas sp. TBZ9 and Marinobacter sp. TBZ23, Isolated from Urmia Lake**

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Background and Aim: Iron is an important abundant element in the environment which its mobility and stability is partly under the control of oxidation state. Microbial iron reduction plays an important role in the iron cycle and affects the exchange of nutrients and trace elements in ecosystems. Dissimolatory reduction of ferric iron oxides plays an important role in mineralization of organic materials in anoxic soils and sediments and also makes up an important fraction of anaerobic carbon metabolism is special marine and freshwater environments. Some bacteria and archaea can grow anaerobically using Fe (III) as the sole electron acceptor and some fungi also are able to reduce Fe (III) to Fe (II).

Methods: In this study, iron reduction was investigated using Halomonas sp. TBZ9 and Marinobacter sp. TBZ23, isolated from Urmia Lake in Iran. Differential pulse polarography was exploited to measure different reduced forms of iron in control and treated samples.

Results: Both isolates are able to reduce Fe (III) to Fe (II) and Fe (II) to metallic Fe Based on intensity of differential pulse polarogram peaks observed respectively at -0.34V and -0.51 V, it seems that TBZ23 reduces almost 20% of available Fe (III) to Fe (II) and this reduction rate is about 80% for TBZ9 during 10 days of incubation.The rate of reducing Fe (II) to metallic Fe is seemingly 25% for TBZ23 and 63% for TBZ9 during 10 days of incubation.

Conclusion: TBZ9 can play an important role in environmental cycling of iron, carbon, and other elements.

Keywords: Halomonas, Urmia Lake, Marinobacter, Fe Reduction, Iron, Bacteria

P612: **Application of magnetic fields in Aquaculture**

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Background and Aim: Microbes are a major cause of dysfunction in various industries and dispel industrial system would suffer too expensive.The aquaculture is the one of the important biological industries which several solutions have been proposed for remove or reduce the damage caused by these germs

Methods: One of these ways is the use of magnetic fields and applied it in the aquatic environment.

Results: It is seen that the redox activity of bacteria under magnetic treatment will be decreased and also bacterial DNA stability is lost and the result will be reduction in the number of bacteria. In simultaneously study on gram positive and gram negative bacteria were seen magnetic field as a deterrent factor to growth, have higher effect on gram negative bacteria than gram positive bacteria.

Conclusion: According to this theme that the high percentage of aquatic pathogenic bacteria is classified as gram negative bacteria, magnetic field can be applied as the way for remove aquatic pathogenic bacteria.

Keywords: Aquaculture, magnetic fields, negative germs

P613: **Evaluation of Bacterial Communitiy in the Rhizosphere Soil of Vetiver (Vetiveria Zizanioides (L.) Nash) at Different Growth Stages by PCR-DGGE**

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Background and Aim: Vetiver is a plant of Poaceae family and due to its unique morphological, physiological and ecological properties, it is used for soil and water conservation, particularly in phytoremediation, soil erosion control, slope stabilization.

Methods: Recently, molecular approaches based on the analysis of 16S rDNA sequences have been applied to the assessment of microbial diversity in environmental samples. In this study, soil samples were collected from rhizospher soil of Vetiveria Zizanioides (L.) Nash and molecular approaches (PCR-DGGE: polymerase chain reaction - denaturing gradient gel electrophoresis) and sequencing were used.

Results: The findings of this study demonstrated predominant rhizospheric bacterial community at different growth stages of the plant in the pilot scale.

Conclusion: This technique provides an insight into the bacterial community structure that was associated with rhizosphere soil of vetiver.

Keywords: Bacterial communities, Rhizosphere, Vetiveria zizanioides (L.) Nash, Rhizospher, PCR-DGGE.

P614: **Monitoring**

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Background and Aim: Iran

Methods: Recently,

Results: Results

Conclusion: The results

Keywords: microbial diversity, phytoremediation, petroleum hydrocarbons,rhizospher, PCR-DGGE

P615: **Biodiversity of ionizing-radiation resistant bacteria recovered from soil samples in a radioactive site in Iran**

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Background and Aim: Ionizing radiation resistant organisms has been observed in several members of the domains Bacteria and Archaea. The aim of this study was to investigate the biodiversity of radiation resistant bacteria in soil samples of Iran.

Methods: Soil samples were collected from the radioactive site in Iran. One gram of soil were exposed to levels of radiation between 0 and 30kGy at a dose of 3.19 Gy/sec at room temperature using a Gamma Cell model GC-220 60Co irradiator. After radiation, the soil samples plated in Tryptone Glucose Yeast Extract (TGY) agar and incubated at 30 C for 5 days. All bacterial isolates were identified by standard microbiological and biochemical methods.

Results: Among a wide variety of bacteria, two isolates could withstand to radiation. The survived bacteria were belonged to Micrococcus genus according to microbiological and biochemical features. These strains are aerobic, mesophilic, yellow colony, catalase positive, non motile, coccus and Gram positive.

Conclusion: Isolation of radiation resistant bacteria is important in Microbiology and these bacteria could also be a candidate for bioremediation of radioactive waste sites that contain hazardous mixtures of radio-nuclides, heavy metals, and other toxic chemicals.

Keywords: Microbial diversity, Screening, Radiation resistant.

P616: **Bio removal of lead by Bacillus sp isolated from oil contaminated soils, Khuzestan**

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Background and Aim: In the past decade, enter the pollutants such as heavy metals have been increased in vital ecosystem. That is serious danger for life on the Earth. Heavy metals in most parts of the world are pollutants in various physicochemical forms and should purify. In this research we evaluate some methods for biological treatment for lead removal by related resistant strains.

Methods: To conduct the study, five samples of oil contaminated soils of Khuzestan zones were collected under sterile conditions and transferred to laboratory immediately. The soil samples were homogenized and diluted by sterile saline till to 10-10 and cultured on Tripticase soy agar containing 5ppm lead nitrate. Resistant strains were isolated after 24 hours of incubation. Isolated bacteria were identified by biochemical tests. Then, the MIC test was used for screening of resistant strains on Tripticase soy agar.

Results: Absorption tests of metal were showed 98.16% removal of lead in top strain by atomic spectroscopy analysis.

Conclusion: In conclusion, this study reveals the significance of using the Bacillus sp in the bioremediation of Pb contaminated soil.

Keywords: bio removal, lead, Bacillus, oil contaminated soil

P617: **Biosorption of lead by Pseudomonas sp isolated from Khuzestan's Petroleum soils**

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Background and Aim: The contamination of the environment by heavy metals is a major global concern because of their toxicity and a threat to human life and environment. In this research we evaluate some methods for biological treatment for lead removal by related resistant strains.

Methods: Hence, five samples of oil contaminated soils of Khuzestan region were collected and transferred to laboratory up to 24h. The soil samples were homogenized and diluted by sterile saline till to 10-10 and cultured on Luria Bertani agar medium containing 5ppm lead nitrate. Resistant strains were isolated after 24 hours of incubation. For isolation of appropriate gram negative strains the samples cultured on Macconkey agar. Isolated bacteria were identified by biochemical tests.

Results: From total of 24 strains of isolated Pseudomonas, 10 strains were resistant to lead by MIC method. Absorption tests were showed 91.79% of metal removal from aqueous solution by top strain.

Conclusion: In conclusion, this study reveals the significance of using the Pseudomonas sp in the bioremediation of Pb contaminated soil.

Keywords: bio sorption, lead, Pseudomonas, oil contaminated soil

P618: **Liquid Paraffin Degrader- Bacteria Isolated from Several Soils of East- Azarbayjan, Iran**

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Background and Aim: Biodegradation is an efficient, economic and environmentally safe treatment technique. By regarding that micro flora of soils are very diverse and it differ from one site to others. The objective of this study was to isolates the microorganisms with liquid paraffin degradation potential from different soil samples that were collected from different cities of Iran.

Methods: 55 colonies of different bacteria were isolated from soil samples that collected from Jolfa, Tabriz, Azar shar, Meyaneh, Marageh, and Sarab cities of Iran. The ability of degradation in all isolates was studied and the produced metabolites were identified with GC mass analysis.

Results: The 8 (14.5%) different colonies degrade more than 70% of liquid paraffin and different metabolites were produced.

Conclusion: This research might be to optimize the process for molecular identification of strains of microorganisms which are found to have degradative capabilities and assay their abilities in field.

Keywords: GC mass, Bio degradation, Soil, East Azarbijan

P619: **Biological control of plant pathogenic fungi using iturin-producing Bacillus subtilis isolated from domestic soil compaired with standard strain**

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Background and Aim: soil-born plant pathogens are a major problem in crop production. Synthetic chemical fungicide can cause environmental pollution and induce pathogen resistance. biological control of plant diseases by microorganisms is suitable alternative to use of chemical fungicide. strains of Bacillus subtilis can play a major role in plant disease suppression through various mechanisms including the production of secondary metabolites. lipopeptide antibiotics synthesized by B. subtilis including iturin family are amphiphilic cyclic peptides and show wide spectrum of antimicrobial activity against bacteria and fungi through increase membrane permeability. The aim of present investigation was to examine the potency of iturin A production from the Bacillus subtilis isolated from domestic soils (Tehran), and test their antifungal activity against Pythium ultimum, Fusarium solani compared with Bacillus subtilis ATCC 21556.

Methods: Eight soil samples were collected and B. subtilis were isolated from each soil sample. The isolates were screened by antifungal activity and the culture conditions were optimized. The bacterial metabolites were then obtained from 4 days grown isolates, purified and were confirmed existence of iturin by chromatography method. The iturin A (Sigma) and ATCC 21556 were used as standards.

Results: Total, 160 were isolated from soil samples which 38 spp. were confirmed as B. subtilis. three spp were then identified as the most antifungal active strains. Then nutrient broth with carbon and nitrogen sources glucose, yeast extract, neutral pH and 30°C incubation temperature were optimized for best production. The HPLC results showed the extent of iturin A for three isolates B. subtilis compared with ATCC 21556. One isolate identified as Bacillus subtilis 102 showed the best antifungal (iturin A) production.

Conclusion: We conclude end homeland domestic isolates of B. subtilis have considerable antifungal activity when compared with standards. Therefore, they could be suitable candidate for biological control plant pathogen and can be used as biopesticides for plant protection and also useful substituted for chemical fungicide.

Keywords: Biologicl control, Bacillus subtilis, Domestic strain, IturinA, Pythium ultimum, Fusarium solani

P620: **An assay on citrinin toxin production and quantity in the Aspergillus isolates of northern Iran, using ELISA technique**

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Background and Aim: Aspergillus is the most important toxin-producing fungus and abundant in habitats of northern Iran, which is considered as the main source of food is present. As far as the production of Citrinin toxin by Aspergillus have not been widely studied, so as to include multiple species and how the process of production and secretion of this toxin in Aspergillus is unknown, this research is conducted.

Methods: In this study, fifteen species of Aspergillus fungal isolates in Czapelc medium containing 2% malt extract with around 200 RPM at room temperature for a week, then isolation and filtration fungal populations by medium in order to measurement of the amount of toxin by the competitive ELISA, with dry fungal biomass utilization 2gr on the desiccator with physical comminution (grinding) of cells (centrifugation with Glass Pearl), and then. Methanol and Estonia extraction of biomass to measure the amount of toxin by competitive ELISA (r-biopharm: lRidascreen Fast Citrinin) is used.

Results: By increasing fungal infection and related damages, the motivation of microbiologists has increased to fungal contamination in human habitats. Because these toxins are not easily recognizable, study of their characteristics is very important. According to the survey results, should be in terms of food contaminated with Aspergillus fungal toxin Citrinin like there other Aspergillus toxins studied to determine its health.

Conclusion: This study showed that Aspergillus can produce Citrinin toxins are native to northern Iran, Citrinin toxin production in different species difference is significant. Average rate of toxin production in isolates from 0 to 2009.29 ppb is mutable. The highest number of investigated isolates has been produced toxin in the range of 460.93 ppb average.

Keywords: Aspergillus, Citrinin, ELISA.

P621: **Measuring changes Citrinin toxin produced by Aspergillus species under the effect of different amounts of olive oil, fruits**

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Background and Aim: The Second Microtoxines are fungus which is famous as mould. The fungus which produce toxins grow up on different types of Sustras such as grains and by-products of grains that may be eaten by pets. Lots of these Microtoxines are very strong toxins that may affect on humen, animals and birds. citrinin is a fungus metabolist which is made of penicilium and ampergillus. These fungus grow up on grains and rotten organic materials, because of that they are known as a storage fungus.

Methods: The 5cm plates are made by fruit olive oil in cultivation CDME 2% (CHAPEK AGAR 10%+4 lit dextrose+ 5%malt extract with 5%, 10%, 20%, 40% thickness which are cultured with maximum citrinin toxin in the same plates. All plates were warmed in 25±2 degrees and after 30days extraction by and the results will be done by direct competition Elisa.

Results: According to data from growing up of known colonies of Ampergillus in standard situation with CHAPEK AGAR base, and comparing between speed and amount of growing up (colony diameter) and details of morphology of known colonies from Aspergillus in the standard lab situation which is based on the known characteristics, we realized that speed of growing of the isolated ones was reduced 50-100% which wasn't the same in all kinds. According to apparent details of a morphologic, we realized amount of fruit olive oil in lab cultivation, had a different impacts on each of them.

Conclusion: According to information about metabolism of fat acids in Aspergillus cells, we may conclude that diversity of fat acids in fruit olive oil caused those changes. We may be able to use fat acids which are affective on reducing Aspergillus as food preserver. Of course it needs more research and we shouldn't forget that metabolism of fat acids in producing cycle interferes on metabolism of the second Aspergillus such as toxins. We should expect producing of known toxins have been affected and are known as a an important method in reducing or stopping of toxin production which is under survey in following. Keybord: olive oil citrinin aspergillus

Keywords: citrinin,Aspergillus,olive oil,Elisa

P622: **The characterization of new B.ssp.tonekaboneins strain lipase producing, isolated from hot spring in north of Iran**

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Background and Aim: Lipase enzyme is an important group of biocatalyst for biotechnological application. In particular, thermostable lipases from thermophilic bacteria have the potential to play important roles in industrial applications because they possess relatively high thermodynamic stability both at elevated temperatures and in organic solvents .purpose: in this experiment, the produced amount of lipase enzyme, the characteristic of biochemical strain, and resistive stability toward heat and PH level were studied.

Methods: Material and method: the colony of thermophilic bacteria was separated from BTM AGAR environment .Then, the halo of separated lipase in the BTM environment which contains rhodamine and tree glisrid butyrate were studied .in the end , the stronger positive samples were separated from positive samples through 16srRNA test and their biochemical characteristic was studied through API50CHB test .the produced amount of lipase enzyme , resistive stability toward heat (50°C,55°C,60°C,65°C), PH (6,7, 8, 9 ,10), and resistivity of enzyme in temperature (50°C, 60°C,70°C, 80°C,90°C) by using of p. nitrophenyl palmitate were studied.

Results: Result: in this study, the new strain of thermophilic bacteria that produced lipase , named Bacillus .ssp.tonekaboneins was separated . This bacteria in 10 PH and 50 °C contains the maximum production of lipase enzyme. And Also, in 70 °C temperate has the Max stability. The result is showing that the isolated bacteria from Ramsar's Hot spring could produce a resistive lipase which is also stable toward Basic lipase.

Conclusion: Conclusion: In view of the variety of applications, there has been a renewed interest in the development of source of lipases. Bacteria, yeasts and moulds can produce lipase but the availability of lipase with specific characteristics is still a limiting factor, thus this leads to the search for new lipases amongst the bacteria isolated from different water temperature.

Keywords: Bacillus .ssp.tonekaboneins, hot spring, p. nitrophenyl palmitate , lipase producing

P623: **The characterization of Lactic acid bacteria isolated from contaminated soil with Wastewater of local Yogurt Making from Tonekabon city**

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Background and Aim: Soil, due to the physicochemical conditions, includes a wide variety of different microorganisms with high metabolic potential. Probiotics - live microbial cultures as being consumed for health−bring benefits beyond providing basic nutritional value .Lactic acid bacteria (LAB) the largest group of probiotics and vastly used in industry and health. Lactic acid bacteria have been isolated from specific habitats, including dairy products (and in this study from contaminated soil with local yogurt), plants, sewage, manure humans and animals .The purpose of this study was isolation and analysis of potential probiotic, LAB bacteria whose natural habitats−dairy products−have changed to non-specific habitats such as soil.

Methods: In this research 10 samples of contaminated soil with wastewater of local yogurt were randomly collected from highlands in Tonekabon city(North of Iran). LAB bacteria were isolate using Selective medium MRS and M17 and then identified by API 50CHL tests, also probiotic potential of isolated colonies was evaluated by antibiotic susceptibility, acid tolerance and bile resistant tests. Finally, the identification of strains was verified by 16S rRNA gene sequencing.

Results:: In this study, we could successfully isolate lactobacillus coryniformis, lactobacillus guizhouensis, lactobacillus coryniformis subs pecies torokoent, lactococcus lactis subs pecies lactis, lactococcus garviae, leuconostoc pseudomesenteroides and leuconostoc mesentroides._Consequently, evaluation of isolated colonies showed that lactococus lactis subspecies lactis had highly significant probiotic potential

Conclusion: The survey conducted here demonstrated that contaminated soil with local yogurt can be the source of many lactic acid bacteria.LAB bacteria have high probiotic potential. These bacteria are able to acid tolerance, and bile resistant. More ever, soil microorganisms are highly capable of producing metabolic products. In the environments such as soil, wide spread genetic exchanges take place in microorganisms and extend the ability of producing metabolic products among themselves, some metabolic products include bacteriocin, enzyme and antibiotic. Due to the genetic exchange among soil microorganisms and LAB bacteria in soil environments, there is a hypothesis, which suggests that LAB bacteria obtain the ability of producing metabolic products, and soil bacteria also obtain probiotic potential by transporting elements genetic from LAB bacteria. Thus we can assume that so many genetic exchanges occurred between soil bacteria and LAB isolates. The exchanges cause probiotic potential to increase. Studies have shown that yogurt produced by traditional methods can better carry for lactic acid bacteria, and soil treated to traditional products can coexist with plant roots, to form plants are the source of probiotic which can be useful indirectly for animals and humans.

Keywords: Lactobacillus guizhouensis, , Lactococcus garviae, contaminated soil, yogurt

P624: **Research on macroscopic and electro-microscopic morphology of some native Sinorhizobium meliloti isolates**

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Background and Aim: Rhizobia are Gram-negative bacteria that are symbiotic with plant roots Leguminous to fix atmospheric nitrogen. The species of Sinorhizobium (Ensifer) meliloti is symbiotic with alfalfa that is useful in nutritional for livestock and medical purposes. Use of chemical fertilizers has a lot of environmental and human health risks. The aim of this study was to isolate native Sinorhizobium bacteria coexist with alfalfa and investigate their microscopic, macroscopic, and electro-microscopic properties.

Methods: Bacteria were isolated from the roots of alfalfa plants that were collected from some areas of Khorasan Razavi and examined for catalase, oxidase, motility, melanin and fluorescence tests. The nodulation test was performed in tubes containing agar. Bacterial morphology was evaluated using transmission electron microscopy and acetate uranyl 2% staining.

Results: 10 bacteria were isolated from collected plants. 8 of 10 isolated bacteria developed nodules in the roots of plants grown from seed variety Hamadani. All isolates, produced pigment in tests of melanin and none of them had fluorescence property. All were found to bacilli and coccobacilli Gram-negative bacteria. Their colonies were convex, mucoid and white to cream color. Colonies diameter ranged from 2 to 7 mm. Bacteria were seen polymorphs and forms Y forms, stretched, bent or clubbing in smear of nodules. The shapes of nodules were different in the roots of grown plants. All bacteria have peritrichous multiple flagella in electron micrographs.

Conclusion: The results of this research showed that different isolated Sinorhizobium bacteria were similar in shape and size, and were alike as reported by other researchers. Their shapes of nodules were different on roots of alfalfa plants. Isolation, identification and investigation about Rhizobium features that are native and adapted to soil and climatic conditions of our country can be valuable in replacement of bio-fertilizers instead of chemical fertilizers in various parts of the country and sustainable agriculture.

Keywords: Sinorhizobium meliloti, Electro-microscope, Morphology, Infection test

P625: **Biological control of Pseudomonas syringae and Agrobacterium tumefaciens by Chitosan solution**

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Background and Aim: Such as very damaging bacteria that become yearly cuse to incurable damages of benefit agriculture valued products, can indicate to Pseudomonas syringae and Agrobacterium tumefaciens. Nowadays use of natural methods are benefit methods for control of plant diseases. Chitosan is a non toxic and biodegradable polymer that can replace for expensive and incurable damages of chemical methods. So that aim of this project was usage of chitosan as biocontrol agent of Pseudomonas syringae and Agrobacterium tumefaciens pathogenic bacteria.

Methods: Also two type of Chitosan solutions in densities 4, 5, 6 and 7 milligrams per milliliter were prepared and antibacterial activity of these were studied by drop, disk, cup and microtitr plate methods to determine MIC (Minimal Inhibitory Concentration) and MBC (Minimal Bacteriocide Concentration).

Results: at drop and microtitr plate methods was shown that antibacterial activity of Chitosan against of Pseudomonas syringae and Agrobacterium tumefaciens strains increased to increase of concentration of chitosan solotion, but in disk and cup plate methods was not shown any antibacterial activity.

Conclusion: Results completely indicated that chitosan was a potential bactericide against bacterial pathogens, Pseudomonas syringae and Agrobacterium tumefaciens and its is important factor for biological control of these bacteria that indicated in much papers.

Keywords: Biological control, Chitosan, Pseudomonas syringae, Agrobacterium tumefaciens

P626: **Antibacterial effect of Pseudomonas putida P19 siderophore on Xanthomonas campestris and Pseudomonas syringae**

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Background and Aim: Xanthomonas campestris and Pseudomonas syringae are phytopathogenic bacteria that cause incurable damages to beneficial agricultural products. Pseudomonas putida P19 is produced siderophore. Siderophores are defined as relatively low molecular weight and ferric ion specific chelating agents elaborated by bacteria and fungi growing under low iron stress. Nowadays Siderophors that use in biological control. So that in this study we used Siderophor of Pseudomonas putida P19 as biocontrol of Xanthomonas campestris and Pseudomonas syringae.

Methods: At first, extraction of Siderophore by Meyer and Abdallah method was done. The second antibacterial influences of the Siderophores produced by Pseudomonas putida P19 were studied aftdr 24, 40 and 72 hours by disk, cup and microtitr plate methods to determine MIC (Minimal Inhibitory Concentration) and MBC (Minimal Bacteriocide Concentration).

Results: The results was shown that any antibacterial influences of produced Siderophore in disk and cup plate on Xanthomonas campestris and Pseudomonas syringae but strong antibacterial effect on Xanthomonas campestris in microtitr plate (the emount of MIC is 0.00124 and MBC is 0/036) and any effect on Pseudomonas syringae.

Conclusion: Therefore, use of Siderophores for suppression of fungi and other plant pathogens have been reported previously, although few studies reported such on bacteria.

Keywords: Antibacterial, Pseudomonas putida P19, siderophore, Xanthomonas campestris Pseudomonas syringae

P627: **Antibacterial activity studing of prodigiosin pigment against of Xanthomonas campestris and Erwinia carotovora**

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Background and Aim: Today experts have conducted numerous studies to control plant diseases in order to preserve quality, food abundance, appropriate nutrition and the food required by human being through the plants that are being produced in the world. During recent years, it has been remarkably important to biologicaly control the plant pathologic factors. For this purpose in the present research we have used secondary metabolit of Prodigiosin (by Serattia marcescense bacteria PTCC1111), for biocontrol of Xanthomonas campestris and Erwinia carotovora plant pathogens. prodigiosin wich is a red pigment and has antibacterial, antifungal and et al activities that is used in study.

Methods: at First the influence of various factors on the amount of production of prodigiosin pigment was studied and then pigment produced in powdered peanut. Extraction of pigment was made by acidic method and purified by column chromatography and then identified with thin-layer chromatography , visible and UV spectrophotometric, and NMR methods, at last densities of 0.05, 0.15 and 0.5 milligrams per milliliters purified pigment in HCL4% one molar was prepared and to study its antibacterial effect on Pseudomonas syringae phytopathogenic bacteri were used by disk and microtitre plate to determine MIC (Minimal Inhibitory Concentration) and MBC (Minimal Bacteriocide Concentration).

Results: The results was shown that in disk plate Method was not shown any results and in microtitre plate method minimal inhibitory and bacteriocide concentration of purified pigment were equal to 0.00312 milligrams per milliliters for Erwinia carotovora and 0.00156 for Xanthomonas campestris.

Conclusion: To present was considered prodigiosin pigment effects on pathogenic bacteria in human and animal and was studied very much on phytopathogenic fungi. Effects of this pigment were not studied or so little on phytopathogenic bacteria.

Keywords: prodigiosin pigment, Serratia marcescens, Xanthomonas campestris, Erwinia carotovora

P628: **Molecular screening of nep like genes in actinomycetes isolated from soils of Iran**

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Background and Aim: Necrosis and ethylene inducing peptide (Nep) like family of proteins (NLPs) are a novel group of phytotoxins introduced during the last two decades. nlp genes have high prevalence among fungi, oomycetes, Gram positive and Gram negative bacteria and cause necrotic lesions in dicots. In this study, we have detected the presence of nlp genes by molecular approach in actinomycetes isolated from soils of Iran

Methods: Soil samples were collected from rhizosphere and philosphere of the plants which were suspected to disease in different provinces of Iran. Actinomycetes were isolated and purified on GAP and ISP2 mediums, respectively and preserved in University of Tehran Microorganisms Collection (UTMC). For primary screening of necrosis causing actinomycetes, fermentation broth of the entire strains were prepared and were used for assaying the herbicidal activity against disinfected sunflower (Helianthus annuus) leaves in 1.5% water-agar plates. All the assays were performed in triplicates and non-inoculated culture medium was used as the negative control. During secondary screening, selected isolates were subjected to genomic DNA extraction and PCR amplification of the nlp gene using a pair of specific degenerative primers with the following sequence: nlp forward: 5" CgACTACgACVVSgACggCTg"3, nlp reverse: 5"gTTCTCSggSggYTCgTC"3. The primers had been designed based on homologue fragments of seven registered actinomycetes nlp genes in Genbank database using multiple alignments (ClustalW2). PCR products were ligated in SmaI digested pUC19 plasmid and transformed in E.coliXL1-blue. The presence of nlp genes was confirmed by Colony PCR and sequencing. Finally, phylogenic characterization of the final selected isolates was performed by PCR amplification of 16SrDNA genes and sequencing the generated products.

Results: Actinomycete isolates (100) were purified from soil samples. Herbicidal activity assaying of these strains resulted in necrosis and chlorosis of sunflower leaves by spraying the fermentation broth of 25 isolates whereas the remaining caused no lesions in the leaves, the same as negative control. PCR amplification of the DNA of the positive isolates with nlp gene primers showed ~400bp bands in 12 strains. After sequencing of the cloned PCR products, it was revealed that all of them were belonged to NPP1 (Necrosis-inducing Phytophtora Protein 1) domain. Amino acid analysis of the obtained sequences showed that actinomycetes nlp genes are in typeII group of nlp genes because the W residue in the heptapeptide motif ''GHRHDWE'' is not completely conserved among all. Phylogenic identification of the strains indicated the presence of nlp genes in rare actinomycetes other than the genus Streptomyces.

Conclusion: In this report, we have designed a couple of specific degenerated primers to screen nlp genes in actinomycetes isolates. Our results show that nlp genes have high occurrence among various genus of this branch of bacteria.

Keywords: Actinomycetes, necrosis and ethylene inducing protein, molecular screening, phytotoxin

P629: **Identification of Symbiodinium clades and subclades in reef-building corals off Hengam Island (Persian Gulf-Iran) by PCR-DGGE**

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Background and Aim: Reef building corals associate with unicellular symbionts which belong to genus Symbiodinium. Up until now, 9 clades of Symbiodinium have been identified using molecular method. Clade D is the most tolerant type among the all clades have been identified so far. However, the type of coral has important role in the resistance clade.

Methods: Purpose of this study is identification of Symbiodinium clade in depth off Hengam Island. Samples were collected from depth of 5-7 m in Hengam Island including: Cyphastrea microphthalma, siderastrea savignyana. Specimens were kept in 20% DMSO until were transferred in the laboratory. DNA was extracted with CTAB/chloroform method. Using a combination of the internal transcribed spacer-2 and Denaturing Gradient Gel Electrophoresis, the cladal and subcladal variability of Symbiodinium were assessed.

Results: The sequencing results indicate that there are two different clades and two subclades of clade D in depth including: Clade D in S.savignyana and C.microphthalma, Subclades D1 and D1a in C. microphthalma.

Conclusion: As clade D is a more therrmal tolerance clade among other clades of Symbiodinium, dominance of this clade in northern parts of the Persian Gulf may be due to high fluctuation in temperature and other stressful conditions of this area.

Keywords: Symbiodinium, Clade, ITS2, Hengam Island, DGGE

P630: **Frequency of the Pathogenic Campylobacter in the Samples of the Various Types of Fishes of Caspian sea**

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Background and Aim: Campylobacter jejuni and C.coli are the leading causes of human bacterial gastroenteritis worldwide. Campylobacter species are primarily zoonotic, with a variety of animals implicated as reservoirs of human infection ,and as mentioned, the most prevalent species are C.jejuni and C.coli. Campylobacter can be transferred to human through the ingestion of contaminated food, and one of the potential sources of infection is seafood such as fishes. The aim of this study was to discover the frequency of this bacterium in the fishes of Caspian Sea and to identify the species of it.

Methods: Sampling was done from fall in 2012 to spring in 2013. Samples were mostly taken from the moucosal membrane of the intestinal system of the fishes. The samples were cultured in the Preston broth base medium and Campylobacter selective agar in 42°c and anaerobic condition for 72 and 48 hours respectively. After the observation of the growth of the bacterium, the PCR technique was used and conducted using specific primers.

Results: Of 154 samples collected from various types of fishes, any of them weren`t infected with the Campylobacter spp. With regard to the results obtained from this research, digestive system of the fishes can not be counted as an appropriate medium for the growth of Campylobacters.

Conclusion: Considering the report of the contamination of this region`s waters with Campylobacter presented by the previous researchers and the lack of this bacterium in the samples of fishes, it can be claimed that digestive system of fishes has not appropriate conditions for the growth of Campylobacters. The reason could be the temperature of the fish`s body that is much lower than the temperature that Campylobacter need for survive and growth, or the low ability to compete with another bacteria in the high competitive condition of the intestinal system.

Keywords: Campylobacter, Fish, Caspian sea, north of Iran

P631: **Isolation and Identification of Nontuberculous Mycobacteria from water and soil in Robat karim by using PCR-RFLP**

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Background and Aim: Nontuberculous mycobacteria are environmental, opportunistic pathogens whose role in human disease is increasingly recognized. Nontuberculous mycobacterium infections are caused by mycobacteria that found in water and soil . Identification of Nontuberculous mycobacteria is important for both clinical and epidemiological studies because of the worldwide propagation of these organisms. In this study the prevalence of Nontuberculous mycobacteria from water and soil in different part of Robat Karim was determined by using PCR-RFLP of the hsp65 gene and 16S-23SrRNA.

Methods: In this study a total of 454 water and soil samples were collected from different areas of Robat Karim.After decontamination of isolates, they were cultured onto Lowenstein Jensen media.hsp65 gene amplified and then digested by two restriction enzymes, BsteII and HaeIII.Digested products were analyzed using polyacrilamid gel electrophoresis (PAGE).Also16S-23S rRNA, gene amplified and then digested by two restriction enzymes, HaeIII and CfoI. Digested products were analyzed using PAGE.

Results: Based on the culture and microscopy observation, 49 (21.5%) samples of 227 soil samples and 144 (63%) samples of 227 water samples were positive regarding mycobacterium. By using PCR-RFLP, 54 water samples,7 soil samples were contained slow growing mycobacteria and 29 water ,7 soil samples were contained rapid growing mycobacteria.The results achieved by molecular method match completely with that of culture. Mycobacterium tuberculosis complex with 18.5% frequency was the predominant isolated mycobacteria in water and soil samples.

Conclusion: Mycobacterium tuberculosis complex was the main mycobacterium isolated in the total of soil and water samples in Robat Karim region.

Keywords: Nontuberculous mycobacteria, PCR-RFLP, 16S-23S rRNA gene, hsp65 gene

P632: **The survey of rate of fluorene degradation by isolated bacteria from oil-contaminated soils of Masjed Soleiman**

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Background and Aim: Fluorene is a tricyclic PAH. Because fluorene is present in most PAHs mixtures and its structure is found in several mutagenic and carcinogenic PAHs, it is often used as a model structure. The aim of this study, was isolation and evaluation of degradation potential of fluorene-degrading bacteria from oil-contaminated soils.

Methods: . Isolation of bacte¬ria ca¬r¬r¬ied out by means of selective enrichment with an initial concentration of 100ppm of fluorene in liquid BSM as sole carbon source, and the transmission on BSM-agar medium.

Results: . In this study, six isolates were isolated and isolates of F2 and F3 were selected as resistent isolates to high concentrations of fluorene. Biodegradation rates of fluorene were determined by HPLC. By the isolates of F2 and F3, 43.8% and 92% of fluorene was degraded, respectively in liquid-BSM with an initial concentration of 100ppm of fluorene as sole carbon source during fiftheen da¬y¬s of incu¬b¬a¬tion. Using of bioc¬h¬e¬m¬ical tests in order to identifi¬c¬a¬ti¬o¬n of high efficiency isolate, isolate of F3 was gram positive bacilli, catalase positive and oxidase negative, producing spore that belonged to bacillus genus

Conclusion: The isolate of F3 could remove a high rate of fluorene that can be used in order to bioremediation of fluorene in environment.

Keywords: Fluorene, Selective enrichment, HPLC, Bioremediation

P633: **Isolation anthracene-degrading bacteria from oil-contaminated soils of Masjed Soleiman**

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Background and Aim: Anthracene is a three ring PAH containing relatively serious toxicity that as anthracene enters the body, it appears to target the skin, stomach, intestines and lymphatic system and it is a probable inducer of tumors. PAHs, such as anthracene, are natural or anthropogenic constituted from incomplete combustion of organic and from petrochemical industries and oil refinery activities. The main objective of the present work was to isolate and characterize anthracene-degrading bacteria from oil-contaminated soils of Masjed Soleim¬an.

Methods: Isolation of bacte¬ria ca¬r¬r¬ied out by means of selective enrichment with an initial concentration of 100ppm of anthracene in liquid BSM as sole carbon source and incubation at 30°C for six weeks in the dark.

Results: The four isolates(A1-A4) was isolated. In order to selection of high efficiency isolates, concentration of anthracene was increased to 800ppm on BSM-agar. Finally, two isolates of A1 and A3 were selected and using biochemical tests, , isolate of A1 identified as gram negative bacilli, catalase positive and oxid¬ase nega¬tive that belonged to Stenotrop¬hom¬o¬n¬¬¬as gen¬u¬s, isolate of A3 was gram negative bacilli, catalase positive and oxidase pos¬itive that belonged to psuedomonas genus

Conclusion: Selection of high efficiency isolates can be used for cleaning up of high levels of contamination in soil.

Keywords: Anthracene, Degrading bacteia, PAHs, Bioremediation

P634: **The Inhibitory Effect of Alfalfa germinated seed on Bacilli Chemo Taxis**

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Background and Aim: Goal: In the present study the inhibitory effect of germinating Alfalfa's(Medicago sativa) seed on Bacilli chemo taxis is investigated.

Methods: Method: In order to survey chemo taxis, bacteria were cultured in Lawn plate. For this reason, the Agar-Agar medium containing high percentage of water is utilized. The plant, alfalfa, was used in different states including seeds, germinating seed and the numbers of germinating seeds in a medium. Seeds were disinfected with 70% ethanol and 20% sodium hypochlorite for few minutes. Consequently seeds were washed with sterile water and then they were immersed on the medium under the aseptic condition. The incubation was performed under constant light conditionat the temperature of 25ºC for 15 days.

Results: Results: Observations were investigated as presence of inhibitory zone and its spread, germination quantity and the germination length in the samples. After incubation, bacteria were grown and inhibitory zone was observed around buds. Therefore, it can be inferred that there is a positive relation between spread of chemo tactic halo and buds' length. Moreover,the numbers of germinated seeds was reduced in the presence of the bacteria.

Conclusion: Conclusion: Accordingto production of Lantibiotics as a growth inducer by some species of Bacillaceae, such as Bacillus subtilis, it is expected that this bacterium would have positive chemo taxis towards plant’s bud. Not only these bacteria didn’t have positive chemo taxis, but also buds inhibited the growth of bacterium by production of biocide and flavonoid.The measurement of buds' length and inhibitory zone show that Alfalfa’s bud has an inhibitory effect on bacilli growth; however, it should be mention that this relation can be considered bilateral relationship because of the fact that the presence of bacterium will reduce the germination power and speed in Alfalfa's seed germination. Hence it can be concluded that there is an inverse relation between Alfalfa's bud and bacilli. As we know Alfalfa is used as a drug and by this study has been denoted that plant’s bud has antibacterial effect on bacilli, too. So we can introduce it as a drug in order to treat disease that is caused by bacilli infections.

Keywords: Chemo taxis, Bacilli, Alfalfa's seed

P635: **INFLUENCE Bacterial ACC deaminase ON salt tolerance**

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Background and Aim: Plant hormone ethylene is an important signaling molecule involved in many processes in plants, including germination, flower development, ripening, and reactions to many environmental factors [The formation of ethylene induced by various external factors, including viral infection, injury, drought. Sharply increased ethylene production during stress and tissue damage. Many of the strategies used to increase the yield of crops, are aimed at reducing the amount of ethylene synthesized by the plant. It has been found that many bacteria, stimulating the growth of plants, is synthesized enzyme capable of controlling the level of ethylene in the plant. The enzyme 1-aminocyclopropane-1-carboxylate deaminase, hydrolyzes 1-aminocyclopropane-1-carboxylate - the direct precursor for ethylene biosynthesis in plants .

Methods: Further study was aimed at investigating the effect of bacterial ACC deaminase in the salt tolerance of tomato and wheat. The seeds of wheat and tomatoes were planted in wet soil. After 1 week of growth of seedlings of the same size were selected and transplanted into individual plastic cups of 150 ml. Seedlings were treated with a solution of 172 mM NaCl and 207 mM. After three days of seedling was divided into 4 parts. One portion was treated with 40 ml of bacterial suspension P. mendocina 9-40 , the other part - 40 ml bacterial suspension P. mendocina B-162, the third part - 40 ml of the medium, and the fourth part - 40 mL of deionized water. The results take into account at the end of 5 weeks.

Results: For tomato seedlings following data were obtained: the salt concentration of 172 mM, plants treated with a suspension of bacteria P. mendocina 9-40 , exceeds the length of the stem controlled by 5-15% at the root length to 1-14% by weight based on 9-22%, at a salt concentration 207 mM - the length of the stem at 9-21% in length root at 12-49% by weight based on 34-49%. For wheat seedlings were obtained the following results: at a salt concentration 172 mM, plants treated with a suspension of bacteria P. mendocina 9-40, exceeds the length of the stem controlled by 3-10%, by weight, 5-30%, at a salt concentration 207 mM - the length of the stem at 2-3% by weight to 12-17% . The data obtained shows that plants treated slurry P. mendocina 9-40 , superior control plants on indicators such as the length of the stem, the weight of the plant. At the same between the control grown in minimal medium, and control, grown in rich medium, there are differences related to the enrichment of the soil environment substances that increase resistance to salt stress. The most significant differences are observed in the mass of plants.

Conclusion: this study shows that the bacteria producing the ACC deaminase tomato seedlings increased resistance to salt stress. Bacteria reduce the production plant "stress" of ethylene, which reduces the inhibition of growth of tomato seedlings under high salt concentrations.

Keywords: ACC deaminase, salt tolerance, bacteria

P636: **Investigation of growth-promoting properties of P. mendocina in a in vitro system**

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Background and Aim: t is known that for many of the rhizosphere bacterial PGPR-band capacity for synthesis of ACC deaminase is a key factor that contributes to growth-promoting properties. Proof of this were the experiments of several authors, where it was shown that inactivation of the gene acdS (encoding ACC deaminase) bacteria P. putida GR12-2 significantly reduces the ability of these microorganisms to stimulate the elongation of canola roots. The aim of study was to investigate further the ability of us received in the previous phase of the strain P.mendocina stimulate the growth of crop tomatoes.

Methods: Seeds of the test plants were pre-sterilized solution of KMnO4 (0.05%) for 30 min. Thereafter they were washed with sterile distilled water, placed in a sterile petri dish on filter and filled 10 ml of sterile distilled water. Cups were held overnight at room temperature to swell the seeds. Then, water was removed from the plates, and seeds treated with a suspension of test bacteria at a concentration of 107 cells / ml. For the preparation of suspensions of bacteria are cultured in liquid medium glucose 4X saline for 24 hours at 37 ° C. The results were assessed after five days. Measured biomass grown seedlings separated from seeds. Served as control seeds soaked in sterile distilled water (embodiment C1), growth medium 4X (variant K2) and wild-type bacterial suspension P. mendocina B-1299 (option R3). The need for setting the control group of K2 has been associated with in order to trace the possible impact of mineral components of the environment in which the bacteria were grown on the studied parameters - the length of the stem of the germ, root length and wet biomass plants

Results: tomato seeds treated with a suspension of bacteria P. mendocinaB-1299 for all three indicators exceed the controls (K1, K2 and K3). Thus, the length of the stem sprouts seed experiment (identified by the letter A) is 50% more than in the embodiment of R1, 61.5% more than in the embodiment K2 and 31.2% - than R3. Root length of tomato seedlings in the experiment is 28.6% more than in the control variant K1, 38.5% more than in the K2 and by 16.1% - than in Q3. Raw biomass tomato seedlings in the experiment to 34.1% more than in the control K1 55.3% greater than K2 and 15.7% - than k3.

Conclusion: These results demonstrate that even at the level observed tomato seedling plant growth stimulation in the presence of rhizosphere bacteria P. mendocina and a more pronounced effect is typical for bacteria P. mendocina B-1299, in which the ACC deaminase activity is 2 times higher than that of wild-type bacteria. Earlier, a similar dependence was not seen by anyone. Thus, we first demonstrated the dependence of plant growth promoting action of bacteria on the level of production of their ACC deaminase

Keywords: growth-promoting, P. mendocina, in vitro

P637: **Production of PHB by native Pseudomonas and Alcaligenes spp isolated from Soils in jungle Park Tehran**

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Background and Aim: Synthetic polymers obtained from petrol causes soil pollution, for this reason, PHB has gained importance since it can easily be dismantled in nature.

Methods: In this study, 10 strains of the genus Pseudomonas and 10 strains of the genus Alcaligenes isolated from 10 different soil samples which were taken from forest in Iran. The samples were heated at 80°c for 20min in water bath, than were diluted in sterile water and were plated on BHI Agar media. plates were incubated at 32°c for 24-48h. PHB production by these strains were determind by the spectrophotometric method and confirmed by GC-Mass and FT-IR

Results: PHB production was found in Alcaligenes strains ranged from 25-55%(W/V) and in Pseudomonas strains 30-67/7%(W/V) .PHB was found in all strain, among them them P7 Pseudomonas strain and A4 Alcaligenes strain were capable to produce maximum biopolymer 67.7% and 55% PHB .The best shaker rotation and temperature for P7 and A4 strains are 200rpm and 32°c.

Conclusion:: P7 and A4 strains are capable to produce biopolymer and suggested that in the future these strains can be used as one of the producer of biopolymer. Using of isolated Pseudomonas and Alcaligenes spp in biosynthesing PHB which can make them suitable for applications in the packaging industry and as substitute for hydrocarbon-based plastics.

Keywords: Pseudomonas spp; Alcaligenes spp: PHB; FT-IR;GC-Mass,Soil

P638: **Optimization of PHB Production from native Pseudomonas and Alcaligenes spp isolated from Soils in Jungel Park Tehran**

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Background and Aim: Copolymer Polyhydroxyalkanoat (PHAs) is the most well known degradable product of Poly beta- hydroxybutyrate (PHB).

Methods: In this research, for producing this kind of polyesters, 20 strains of the genus Pseudomonas and Alcaligenes isolated from 10 different soil samples which were taken from Jungel in Iran . The samples were plated on BHI Agar media and pure colonies were studied based on morphology, physiology and biochemical characteristics. The different media parameter such as pH, temperature and shaker rotation were optimized for increased PHB accumulation. then PHB production by these strains were determind by the spectrophotometric method and confirmed by GC-Mass and FT-IR.

Results: PHB production was found in Alcaligenes strains ranged from 25-55%(W/V) and in Pseudomonas strains 30-67/7 %(W/V) .PHB was found in all strain, among them them P7 Pseudomonas strain and A4 Alcaligenes strain were capable to produce maximum biopolymer 67.7% and 55% PHB. Based on different pH, pH 7 was more suitable for increased PHB accumulation by p7 and A4 stains .Based on the effect of different temperature and shaker rotation 32C and 200 rpm were more suitable for increased amount of PHB accumulation. PHB production by these strains were in the Logarithmic phase of their growth.

Conclusion: P7 and A4 strains are capable to produce biopolymer and suggested that in the future these strains can be used as one of the producer of biopolymer.

Keywords: Biodegradable plastic ,PHB ,Pseudomonas spp, Alcaligenes spp, FT-IR, GC-Mass

P639: **Isolation of PAH-degrading bacteria from mangrove surface sediments of Nayband Bay**

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Background and Aim: Polycyclic aromatic hydrocarbons (PAHs) are widespread environmental pollutants. The major sources of their discharge into the environment are the oil and coal industries and the combustion of organic materials. Being very persistent, toxic, and carcinogenic, PAHs are of great environmental concern. Therefore, degradation of PAHs and remediation of contaminated environments have drawn much more attentions recently. The use of native or indigenous microflora for bioremediation is of great interest as it is often more useful and beneficial than commercial inoculum which could be out-competed by indigenous microorganisms. The present study was conducted to isolate the PAHs-degrading bacteria from Nayband- Bay mangrove sediments and to assess fluorene (Flu) and phenanthrene (Phe) biodegradation ability of the isolated strains.

Methods: Aerobic surface sediment samples were collected from the mangrove forests at Nayband Bay-Iran. To enrich PAH-degrading bacterial consortium, Fresh sediments was transferred into the conical flask containing mineral salt medium with Flu-Phe (1000 mg. l-1) as sole carbon. Flask was incubated at 30˚C on a rotary shaker at 140 rpm. This process was repeated for four times to obtain the enriched PAH degrading consortium. At the end of the enrichment, bacterial strains in the consortium were isolated by spreading the 10-fold serially diluted consortium on nutrient agar plates. Purified strains were then identified by 16S rDNA gene sequence. Isolated strains were first cultivated in nutrient broth and then transferred with final optical density (OD600nm) of 0.15 to the MSM + Flu-Phe mix (1000 mg. l-1) and incubated at 30˚C on a rotary shaker at 140 rpm for 10 days. The concentration of Flu and Phe at the end of experiments were determined using GC-FID.

Results: Six gram-negative bacterial strains were isolated from enrichment consortium on nutrient agar plates. Bacterial isolates, namely Marinobacter hydrocarbonoclasticus, Roseovarius pacificus, Pseudidiomarina sediminum and 3 unidentified strains were isolated from enriched consortium, using Flu-Phe as the sole carbon and energy source. Among the isolated strains Marinobacter hydrocarbonoclasticus and Roseovarius pacificus showed the best biodegradation ability of Phe (10.6) and Flu (9.2) respectively.

Conclusion: PAHs degrading activities of the bacterial consortium belonging to the genus Marinobacter and Roseovarius have been reported worldwide. In addition some authors isolated Pseudidomarina sp. from oil polluted marine sediments. Our results showed that, indigenous bacteria from mangrove surface sediments of Nayband Bay have potential to degrade Flu and Phe. Isolated strains showed low biodegradation activity, this could be because of an inappropriate isolation. On the other hand, several studies have shown that biodegradation caused by mixed culture is more effective than those circumstances when pure cultures are used which may be due to a broader enzymatic. Also, microbial biodegradation efficiency has been found to depend on other factors. In essence, assessment of the optimal conditions would make the bioremediation process more effective.

Keywords: bacterial biodegradation, fluorene, phenanthrene

P640: **Determination of bacterial resistance threshold to selenate as a toxicant element for living cells**

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Background and Aim: Microorganisms that are exposed to pollutants in the environment, such as metals/metalloids, have a remarkable ability to fight the metal stress by various mechanisms. It is only recently discovered that microorganisms have been explored as potential biofactories for synthesis of metal/metalloid nanoparticles. Among these elements, selenium is of significance in biology as a key trace element required by most living organisms (e.g. as essential components of antioxidative enzymes and as anticarcinogenic metabolites). Among all inorganic states, elemental selenium (Se(0)) (insoluble form and therefore non-toxic) and selenate and selenite have the most toxicity to living cells. Therefore, the main goals of this research were the assessment the capability of bacterial cells to resistance toward selenate toxic levels and investigation the impacts of selenate solution on bacterial growth pattern.

Methods: In order to access of the main goal of this research, determination of resistance threshold of selected isolate was important. Therefore, following the isolation of the most resistant isolates, resistance level was evaluated using broth dilution method by inoculating bacterial suspension in the tubes containing selenate solution in the range of 32.5-1200mM. At the next stage, impact of high concentration of corresponding anion on the best isolate growth profile studied using the challenge of bacterial cells at the absence and presence of 100mM selenate solution. Incubation was performed at 30°C, 150rpm and at the predefined intervals, cell density was meaured by OD600 determination until stationary phase was reached.

Results: It was obsreved that this isolate could reduce selenate to red elemental selenium during its growth which could be applied for biosynthesiz of nano-selenium. When the selected isolate (belonging to the Klebsiella genus) was grown under oxic conditions, some differences in growth kinetics were observed when we compared the control cultures and cultures containing 100mM, showing that growth of the bacteria was influenced by the presence of selenate ions. Moreover, there was difference in lag time between the control and selenate containing cultures.

Conclusion: Biological systems have a unique ability to be self-organized and synthesize molecules that have highly selective properties. Due to this dual behavior (i.e. high resistance level to toxic concentrations of selenate and the toxicity effects of this anion on bacterial cells), it can be deduced that possible synthesis of the nano-particles may be performed by this isolate. Moreover, with respect to the its toxicity effects these particles may be applied in order to therapeutic purposes.

Keywords: selenate, toxicant element, microorganisms

P641: **Study of active secondary metabolites of Streptomyces levis isolated Qom's soiland their antibacterial effects**

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Background and Aim: Streptomyces levisis a member ofActinomycetes family. Chemical compounds extracted from this bacterium have been used as antibiotics, anti-tumoral, anti-bacterial, anti-fungal, antiviral, anticancer factors. The purpose of this study, was to investigate different metabolites of Streptomyces strains isolated from Qom's soils, Iranand antimicrobial effects of them.

Methods: The soil samples were taken from Qom city inspring of 2009. The isolated bacteria were purified and identifiedby common methods. The metabolites of this bacterium were extracted by 7 different solvents(diethyl ether, dichloromethane, hexane, ethylacetate, chloroform, methanoland water). The antimicrobial effects of metabolites were tested by disk diffusion agar method on gram-positiveand gram- negative microorganisms. The metabolites with antibacterial effects were analyzed by GC/MS

Results: Metabolites produced from diethyl ether solvent affected on gram- positiveand gram-negativestrains and15different compounds identified in the active metabolite of the bacterium. The main ingredients were: Docosanoic acid, methyl ester (11.578%),9-12-octadecadienoic acid (2.961%), 5-Tetracosenois acid, methyl ester (8.389%), Bis (2-ethylhexyl) phthalate (2.153%),and D-alpha-Tocopherol (0.959%).

Conclusion: The results of this study were shown that Docosanoic acid, methyl ester (11.578%) and 5-Tetracosenois acid methyl ester (8.389%), are the main metabolites of Streptomyces levis isolated from Qom's soils. Antimicrobial effects of extracts could be due to the presence of 1, 2-Benzenedicarboxylic acid diisooctyl ester compounds and Bis (2-ethylhexyl) phthalate in the metabolite.

Keywords: Streptomyces Levis, GC/ MS, Antibacterialeffects

P642: **Molecular Identification of Streptomyces spp. with Antibacterial Activity Isolated from East Azerbaijan Soils of Iran**

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Background and Aim: Actinomycetes are the most widely distributed groups of microorganisms in nature. They are attractive bodacious and charming filamentous gram-positive bacteria. Based on several studies among bacteria ،the Actinomycetes are noteworthy as antibiotic producers making three quarters of all known products; the Streptomyces are especially prolific. In the course of screening for antibacterial activities and molecular identification of isolated Streptomyces, we used 16S rDNA gene and RAPD_PCR method

Methods: 310 Actinomycetes were screened from 110 soil samples of North-West regions of Iran. On primary screening ،out of 310 isolates, only 44 showed antibacterial activity against test microorganisms by bilayer inoculation technique that coast performed on agar medium . On secondary screening of 44 isolates, 12 isolates with strong antibacterial activity were selected for secondary metabolite fermentation These metabolites tested by disk diffusion method against test microorganisms namely B.cereus ATCC 1431, E.coli ATCC 1399, S.aureus ATCC 29213, Y.enterocolitica ATCC 35669, L.monocytogenes ATCC 33090, K.pneumonie ATCC 1290, S.flexnary ATCC 1234 .Then for molecular recognition and genetic diversity survey, the genomic DNA of the isolates bacteria was extracted and the 16SrDNA gene was amplificated by PCR technique. After purification, were sequenced by Macrogene Company. The sequences of 16SrDNA was studied based on bioinformatics assays and led to the identification of the species of isolated antibacterial Streptomyces. RAPD technique was used to detect genetic diversity of 12 isolates with strong antibacterial genotype and 2 standard genotype (S.griseus PTCC 1455 and S.coelicolor PTCC 1450).

Results: At results 7 isolates were found to have a broad spectrum of potent activity against all the tested microorganisms. The sequences of the 16SrRNA genes from the strains 5-9-1 and 20-4-1 were showed that the strain 5-9-1 was similar to S.sampsonii with 97% 16s rDNA sequence similarity and strain 20-4-1 was to S.mediolani with 96%.In RAPD technique, four of the primers produced polymorphic bands.Cluaster analysis divided the genotypes into 4 clusters, using Jaccard's similarity coefficient. Additional, relationship was found between genetic and geographical diversity

Conclusion: The results of this research suggest that the samples of different regions in East Azerbaijan potentially have unknown and novel species of Streptomyces which can be exploited for production of novel antibacterial agents

Keywords: East Azerbaijan, Streptomyces, Antibacterial activity, 16SrDNA, RAPD-PCR

P643: **First Study Molecular Identification of streptomyces with Antibacterial Activity on Pathogenic bacteria Isolated from North West Soils mountains**

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Background and Aim: Actinomycetes are the most widely distributed groups of microorganisms in nature. They are attractive bodacious and charming filamentous Gram-positive bacteria. Based on several studies among bacteria, the streptomyces are noteworthy as antibiotic producers making three quarters of all known products; the streptomyces are especially prolific. In the course of screening for antibacterial activities and molecular identification of isolated streptomyces, we used 16S rDNA gene

Methods: Streptomyces were screened from soil samples of East Azerbaijan regions located in the north of Iran. In primary screening used bilayer inoculation technique that coast performed on agar medium against test microorganisms namely B. cereus ATCC 1431, E. coli ATCC 1399, S. aureus ATCC 29213, Y. enterocolitica ATCC 35669. On secondary screening, isolates with strong antibacterial activity were selected for secondary metabolite fermentation. These metabolites tested by disk diffusion method against B. cereus ATCC 1431, E. coli ATCC 1399, S. aureus ATCC 29213, Y. enterocolitica ATCC 35669.Then for molecular recognition and genetic diversity. The genomic DNA of the isolated bacteria were extracted and the 16SrDNA genes were amplified by PCR technique. After purification, were sequenced by Macrogene Company. The sequences of 16SrDNA was studied based on bioinformatic assays and led to the identification of the species level of streptomyces

Results: Three hundred and ten streptomyces were screened out of 110 soil samples that only 44 showed antibacterial activity in primary screening. On secondary screening of 44 isolates, 12 isolates with strong antibacterial activity were selected for secondary metabolite fermentation that 5 isolates were found to have a broad spectrum and potent activity against all the tested microorganisms. The sequences of the 16SrRNA gene from the isolates C91,F51,E45 and G41 revealed that the isolate C91 was similar to S. sampsonii with 97% 16SrDNA sequence similarity, the isolate F51 with 82% to Streptomyces lividans, the isolate E45 with 98% to Streptomyces albogriseolus and the isolate G41 was similar to S. mediolani with 96%.

Conclusion: Results of this research suggest that the soil samples of different regions in East Azerbaijan have potentially unknown and novel species of streptomyces which can be exploited for production of novel antibacterial agents

Keywords: East Azerbaijan, Streptomyces, Antibacterial activity, 16SrDNA

P644: **Cyanobacteria extract as a biofertilizer for medicinal plant, Mentha pipertia L.**

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Background and Aim: The economic value of cyanobacteria is one of the most important issues in the biotechnology studies and in the last decades. Heterocystous cyanobacteria are known as a nitrogen fixing microorganisms. These photosynthetic prokaryotes, are capable of stimulating the growth of higher plants by producing growth stimulating compounds. . The effect of cyanobacterial extract on medicinal plant, Mentha pipertia L., was the main purpose of this study.

Methods: In this study, heterocystous cyanobacteria were isolated from various farm soils from Kermanshah province of Iran. Isolates identified according to morphological characters..After isolation and identification of these taxa, monoalgal cultures carried out by growing the isolates in nitrate free BG-11 medium.. Four isolates, Anabaena vaginicola, Cylindrospermum michailovskoense, Trichormus ellipsosporus and Nostoc calcicola, were used as a biofertilizer in this study.Growth of plants was evaluated by measuring growth parameters such as plant height, root length, dry and fresh weight of plant as well as leaf number after 60 days planting.

Results: . In total 10 species belonging to 6 genera from Nostocaceae family were identified... Statistical analysis showed that there are significant differences in studied parameters as compared to control. Results have showed the significant differences exist in vegetative characters such as plant height and number of leaves as compared to control.

Conclusion: Based on the results of this study heterocystous cyanobacteria can be utilized as biofertilizers but more studies would be required to produce these types of fertilizers.

Keywords: Cyanobacteria, Biofertilizer, Medicinal plant

P645: **Isolation of Nodule-Associated Bacteria from the Alfalfa Root and Their Possible Role in Plant Growth Promotion.**

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Background and Aim: The leguminous plants are symbiotically associated with the rhizobia and this requires active nitrogen fixation. This interaction plays a key role in the agricultural crop production. Plant growth promoting rhizobacteria (PGPR) have beneficial effects on legume growth and some strains enhance legume nodulation and nitrogen fixation by affecting interaction between plant and rhizobia. This study was performed to investigate these nodule-associated bacteria with the alfalfa plant and their physiological effects on it.

Methods: Bacteria isolated from alfalfa roots. The ability of these bacteria to nodulation develops and their nitrogenase activities (by GC) were tested in tubes containing alfalfa plants with solid FP media, after one month. To measure physiological parameters of strains on alfalfa, plants were grown in pots with perlite/ solution FP 50%, for 2 months, and then analyzed for plants shoot length, plant dry weight and nodulation. Molecular characterization was evaluated by PCR technique and sequence analysis of the 16S rDNA.

Results: Experiments were conducted on the plant per isolate and control (with no bacteria) with three replications. Average analysis of data showed that inoculation could active nodules and nitrogenase activity on the alfalfa roots. In case of 21M2 isolate, shoot length and whole plant dry weight increased respectively about 7.5 and 16 times. Molecular characterization of this strain showed genetic similarity with Pseudomonas sp.

Conclusion: Plant growth promoting rhizobacteria (PGPR) use one or more direct or indirect mechanisms to improve the growth and health of plants. These mechanisms can be active simultaneously or independently at different stages of plant growth. Among these are phosphate solubilization, biological nitrogen fixation, improvement of other plant nutrients uptake, and phytohormone production (like indole-3-acetic acid). Enhancement of legume nitrogen fixation by co-inoculation of rhizobia with plant growth promoting rhizobacteria (PGPR) is a way to improve nitrogen availability in sustainable agriculture production systems. Results in this study showed plant growth promotion with respect to increase in plant's shoot length and plant dry weight in case of 21M2. So you can probably use this strain in breeding programs of Alfalfa plants.

Keywords: Alfalfa, Bio-fertilizer, PGPR, Rhizobia

P646: **Isolation of endofit bacteria From the Root Nodules of Alfalfa andTheir Possible Role in Plant Growth Promotion**

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Background and Aim: .

Methods: .

Results: .

Conclusion: .

Keywords: BIOFERTILIZER

P647: **Isolation and Screening of D-Limonene-Resistant Microorganisms from Citrus Waste water of Citrus Processing Plant of Kosar in Ramsar**

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Background and Aim: This study reports the isolation of microorganisms that are resistant to environment containing limonene, the most important residue in the citrus industry, that has been identified to exhibit antibacterial activity.

Methods: Orange peel oil used for the effect of D-limonene, that contains %95.11 limonene. For the isolation, samples collected from strategic places of a citrus processing plant (waste water of citrus processing plant of kosar in ramsar) were used. The samples were inoculate in YM medium containing different amount of limonene and mineral medium containing D -limonene (5%, v/v) in rotary shaker at 30˚C/150rpm for 48h. After this period, a 100 µL sample of the culture broth was transferred to a Petri dish (YM medium). The culture growth was evaluated after 48h at 30ºC and all the strains that presented a satisfactory growth (>30 CFU) were considered resistant to limonene. Finally isolates were analysed by using 16s rRNA sequencing. Also the growth rate of isolates was evaluated at different temperatures and pH levels.

Results: The 76 isolates recovered after 48h were identified as Gram positive bacilli(53), followed by Gram negative bacilli (10), yeasts (7) and Gram positive cocci(5).10 MOs (microorganisms) were resistant to limonene concentrations up to 5% in the medium broth, 23 MOs to 10%, 21 MOs to 20%, 17 MOs to 30%, 5 MOs to 50%. Amongst them 18 were able to grow in mineral medium containing limonene as sole carbon source. And 2 bacteria that were resistant to limonene concentrations up to 70% in the YM broth, were characterized by using 16S rRNA gene. Phylogenetic analysis showed that 16S rRNA sequence of the isolates formed a monophyletic clade with Bacillus cereus and Pseudomonas alkylphenolia. Bacillus cereus and Pseudomonas alkylphenolia were resistant to temperature equal with 4 to 50 ?C and pH equal with 5.5 to 12.5 and 4 to 12.5, respectively.

Conclusion: The research described in this paper is the initial step for the exploration of flavor compounds production via biotransformation of limonene, a nonexpensive by-product of citrus industry.

Keywords: Limonene, Resistant Microorganisms, industrial by-products, flavor.

P648: **Study on microbial air contamination of different areas in dairy processing plants.**

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Background and Aim: Microbial pollution is a key element of air pollution. Air normally has no particular germs and the found Microorganisms in the air usually enter into the air by the soil, animals and humans. Microorganisms transmitting through the air, is an important factor in their distribution. Airborne contamination in dairy processing plants can result in manufacturing of low quality products with a reduced shelf life. Food contact processing surfaces can support growth of microorganisms and become a contamination particularly susceptible to contamination by airborne microorganisms. This causes theair microbial flora of the dairy industry and the role of environmental factors is important. This study was aimed to determine microbial contamination of air in different parts of the dairy plants in Shahrekord city.

Methods:: In the study, the microbiological air quality at processing areas was evaluated in two dairy plants by culture settling plate technique which is known as sedimentation technique. The experiment was conducted with a threefold repetition for each microbiological determination. Microbiological analyses included total aerobes, staphylococci, bacillus, and yeasts and molds. In each series of sample, plates containing culture medium were put1m from the wall and every other barrier and they were placed in different areas for 1 hour. After that, samples were transferred to the laboratory for 24 hat 37 ° C to be incubated. After counting colonies, they were identified and isolated based on their shapes and colors under microscope. Then, to identify bacteria, special biochemical tests were used. For evaluation of fungi in the air of Potato dextrose agar plates containing chloramphenicol were used. Grown yeast and mold colonies were counted and recorded. fungi grown were determined using routine laboratory methods including specifying macroscopic and microscopic properties of the teased mount and slide culture technic.

Results: From the total number of colonies isolated in this study, the rate of infection to Gram-positive cocci were 44%, gram-positive bacilli 53/1%, gram-negative bacilli 7/2% and gram-negative cocci 0/57%. From isolated bacteria, the most bacterial species were Bacillus cereus,Staphylococcus aureus, and Escherichia coli, respectively. And from total number of 313 different isolated fungal colonies,60/3% of yeasts,14/6% of Trichosporon, Paecilomyces7/6 %, Geotrichum 5/7%, Madurella 5/7%, Cladosporium 2/5%, Aspergillus 2/2%, Monillia 1% and Rhizopus 1% were counted.

Conclusion:: Because the shelf life of food products were reduced by airborne contamination and it directs to transmitting spoilage organisms and also it causes some diseases if they are pathogens, then necessary precautions should be taken to prevent the transmission of airborne contamination to the product during and after processing.

Keywords: Air borne microorganisms, bacteria,fungi,dairy plants

P649: **Growth kinetic and biosurfactant production by a bacterial strain isolated from oil-polluted soils in Khark Island, Iran**

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Background and Aim: Biosurfactants are amphiphilic compounds consisting of hydrophobic and hydrophilic domains. Hydrocarbon degrading bacteria are usually capable of producing these compounds. In this study, bacterial strain PM04, with high ability to produce biosurfactant was isolated from oil contaminated soils in khark Island, Iran.

Methods: Growth kinetic and biosurfactant production evaluation was performed by colony count, and petroleum and hexadecane emulsification method, respectively.

Results: Results showed that the growth curve of strain PM04 in beet molasses medium (%1) reaches the logarithmic phase after 10.5 hours which will last till 51 hours of incubation at 35 0 C and 150 rpm shaking. However biosurfactant production reached its peak in stationary phase at 63 hours, suggesting that biosurfactant production is one of the secondary metabolite. The maximum special growth rate (µm) and the generation time of this strain during logarithmic phase of growth were calculated as 0.31 (h-1) and 2.18 (h), respectively. Also it was observed that the excreted biosurfactant of this strain keeps its activity in 0-10,000 (ppm) salinity range

Conclusion: These results introduced this biosurfactant as a potential candidate for industrial applications.

Keywords: Biosurfactant, Emulsification, Genatation Time, Growth Kinetice

P650: **Efficient biosurfactant production by a newly characterized thermophilic bacterium Aneurinibacillus aerothermophilus AS01 isolated from urban wastes**

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Background and Aim: To screen and identify biosurfactant producers from urban wastes; testing selected bacterial strain for its ability to produce biosurfactant; measuring surface tension, emulsification activity, bacterial growth kinetics and finally preliminary characterization of extracted biosurfactant.

Methods: Samples were collected from urban wastes in Kahrizak site of Tehran. Screening of biosurfactant-producing bacteria was carried out using a selective medium containing sunflower oil as sole carbon source. Oil spreading test was employed for screening of biosurfactant-producing isolates. The most potent biosurfactant-producing strain was selected and further identified according to the morphological, biochemical and molecular (16S rRNA gene sequence analysis)approaches. Three methods including surface tension (ST), oil spreading test (OST) and emulsification activity (E24%) were employed to evaluate the surface-active properties of biosurfactant. The compositional and FTIR spectroscopic analysis were carried out to elucidate the chemical nature of extracted biosurfactant.

Results: We successfully isolated 120 bacterial strains from different types of urban wastes and found that the thermophilic isolate identified as Aneurinibacillus aerothermophilus AS01 had a high capacity to produce a new lipopeptide-type biosurfactant. It was able to reduce the surface tension of its culture supernatant from 72 to 42 mN/m. The production of the biosurfactant was found to be much higher in medium containing sunflower oil compared to the glucose-containing medium. The maximum emulsifying activity (E24=72%) was attained with toluene and hexadecane and stability of the biosurfactant extended for more than 3 month. The production of biosurfactant and surface tension reduction was parallel to the cell growth. The maximum production of the biosurfactant by Aneurinibacillus aerothermophilus AS01 occurred at a C/N ratio of 2: 1, pH 7.0, temperature 50°C and salt concentration 3%. The analysis of the extracted biosurfactant by thin-layer chromatography (TLC), infrared spectroscopy suggested the chemical structure of a lipopeptide-type biosurfactant.

Conclusion: Biosurfactants have been taken into consideration and investigated reportedly because of their applications in a wide variety of industrial and environmental biotechnology processes. To our knowledge, this is the first report on a lipopeptide-type biosurfactant production by thermophilic bacterium 'Aneurinibacillus aerothermophilus AS01' isolated from urban wastes. Further studies are in progress to reveal the exact structural characteristics of the biosurfactant in details.

Keywords: Biosurfactant; Aneurinibacillus aerothermophilus AS01; Surface tension

P651: **Isolation and identification of Streptomyces from Hot-Springs of Ramsar and their anti-Bacterial potential**

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Background and Aim: The most abundant actinomycetes in nature are Streptomyces. Streptomyces have more than 140 species. Their most distinctive feature is that they form aerial branched mycelium and substrate filaments . Streptomyces are naturally occurring below the surface of the soil, water and decomposed organic matter in Hot-springs (Zinsser's Microbiology). Many strains produce one or more antibiotics. They are Gram-positive but not acid-alcohol fast. Their optimum growth temperature is 25-35°C; and also some species grow at temperatures within Psychrophilic and thermophilic range. Optimum pH range for growth is 6.5 to 8 . They have a relatively slow growth as their bacterial colony can be seen after a few days. A Few species are pathogenic for animals and man and others are phytopathogens. It's very difficult to isolate and identify species of this genus.(Bergey's Manual)

Methods: Water samples were collected from Hot-springs in Ramsar. Streptomyces were isolated and purified from these samples by selective methods and SCA (Starch Casein Agar) (Balakrishnan et.al, 2003 and Rifi et.al, 2004) and AIA (Actinomycete Isolation Agar) media (Jayashree et.al,1991) and Bilayer culture method . They were classified based on nature and morphology. Cross-streak method was used for 5 pathogen species (Egorov,1985). The Anti-bacterial active substance was extractable in ethyl acetate (Westley et.al, 1979. And Liu et.al , 1986). Bacterial supernatant was collected and were screened by agar diffusion method include agar well diffusion method and disk diffusion method (Sen.et.al,1995). Detection and final approval of the isolates was carried out by 16srDNA analysis .

Results: After enrichment of samples in SCB , medium and their culture on agar containing SCA and AIA, seven colonies similar to Actinomycets colonies were identified and isolated (Fig.1). Then, Cross-streak method test was conducted and reference pathogens screend to determin the anti-microbial potential of colonies (Fig.2). At last one colony was chosen and disk diffusion method and well diffusion method were conducted on the metabolits produce from these isolates (Fig.3).With regard to active bacteria producing isolated and purified antibacterial metabolites, Streptomyces.sp, showed broad spectrum of antibacterial activities on gram positive bacteria . The extract was active against bacteria including Bacillus subtilis , Escheriachia coli, Staphylococcus epidermithis, Bacillus cereus. The effect of temperature on the growth and production of metabolits were also studied (Table1). Finally, the isolated bacteria were sequenced by 16srDNA .

Conclusion: The study showed that Hot-spring exhibit diverse bacteria and it served as potential reservoirs for bacteria of antimicrobial activity. We could use this important reservoir for isolation and identification of new strains which produce antibacterial.

Keywords: Antibacterial activity, Hot-springs, Streptomyces , AIA , SCA.

P652: **Evaluation of probiotic potential tests in Lactic Acid Bacteria isolated from breast-fed infants feces**

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Background and Aim: Human milk is a rich source of Lactic Acid Bacteria. These bacteria enter and colonize in infants gastrointestinal tract and found in their feces .the purpose of this study was to isolate Lactic Acid Bacteria from feces of breast-fed infants and evaluate probiotic potential tests in these bacteria

Methods: feces samples were collected and cultured in MRS and M17 media. Then, Lactic Acid Bacteria were isolated and identified according to their morphology, microscopic shape some biochemical tests and PCR, and finally they were evaluated by probiotic potential tests such as: bile and acid tolerance.

Results: the isolated bacteria were: Enterococcus faecium, Enterococcus raffinosus and Enterococcus.sp.pfc4 and all of them showed good resistance to bile salt and acidic pH

Conclusion: the isolated Lactic Acid Bacteria were resistant to bile and acid in gastrointestinal tract and it shows that they have characteristics of probiotic bacteria

Keywords: Probiotic,Lactic Acid Bacteria,Infans feces,bile and acid tolerance,gastrointestinal tract,

P653: **Antagonistic effects of Lactic acid Bacteria isolated from infants feces against Enteropathogenic and Enteroinvasive Escherichia colie**

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Background and Aim: Lactic Acid Bacteria in breast-fed infants intestine have an special rule on host by their metabolic activities. They also protect the host against colonization of pathogens and stimulate the immune system. The aim of this study was to evaluate antagonistic characteristics of lactic acid bacteria from infant feces against enteric pathogens Enteroinvasive Escherichia coli (EIEC) and Enteropathogenic Escherichia coli (EPEC).

Methods: Lactic Acid Bacteria were isolated from MRS and M17 media and identified according to their morphology, biochemical tests and PCR. Then, their antagonistic effects against EIEC and EPEC was evaluated by well diffusion method.

Results: isolated Lactic Acid Bacteria include Enterococcus faecium and Enterococcus raffinosus showed good antagonistic effects against EIEC and EPEC

Conclusion: antagonistic effects of Lactic Acid Bacteria against enteric pathogens proves their good potential in prevention of gastrointestinal diseases.so, they can be used via or replacement of antibiotics in prevention of these diseases

Keywords: antagonistic effects, Lactic Acid Bacteria,probiotic,infants feces,EIEC,EPEC

P654: **An evaluation of mycorrhizal status in Yeddi Göz region, near Naqadeh (West Azarbayjan ,Iran) at two different seasons**

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Background and Aim: Mycorrhizal fungi are important symbionts in plants and have key role in nutrients acquisition and enhancing plant tolerance against environmental steresses. Due to the effects of mycorrhiza on vegetation and geographical spreading of plants, the aim of this survey was to compare mycorrizal state in Yeddi Göz region located in Naqadeh (Iran) during spring and summer in 2012.

Methods: Five species belonged to five families were selected. Samples were carried out from plant roots and their rhizospheric soil in two seasons. . Determine the percentage of mycorrhizal symbiosis was performed using a Phillips and Hayman. soil samples were used to isolate mycorrhizal spores by sucrose density centrifugation method.

Results: The highest percentage of root colonization and spore number was observed in Bromus faciculatus and the lowest percentage of root colonization and spore number was observed in Vaccaria pyramidata .

Conclusion: The results showed a significant positive correlation between root colonization percentage and spore number in both seasons.

Keywords: Arbuscular mycorrhizal fungi, spore number ,percentage of colonization.naqadeh

P655: **An evaluation of mycorrhizal status and its relationship with soil available phosphorus in Soltan Yaghoub region, near Naqadeh (West Azarbayjan Iran) at two different seasons**

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Background and Aim: Mycorrhizae are one of the most useful symbioses which both host plant and its fungi partner get benefit. For determination of Mycorrhizal state, Sultan Yaghoub region located in Naqadeh (Iran) was selected as study area. The aim of this survey was to compare mycorrhizal status in spring and summer in 2012.

Methods: Five species belonged to five families were selected. Samples were carried out from plant roots and their rhizospheric soil in two seasons.Also several soil samples in each habitat were collected for determination of soil available phosphorus. Determine the percentage of mycorrhizal symbiosis was performed using a Phillips and Hayman. soil samples were used to isolate mycorrhizal spores by sucrose density centrifugation method.

Results: The highest percentage of root colonization and spore number was observed in Anthemis tinctoria and the lowest percentage of root colonization and spore number was observed in Ziziphora capitata.

Conclusion: The results showed a significant positive correlation between root colonization percentage and spore number in both seasons. Soil analysis showed a significant negative correlation between soil P content and spore number and between P content and root colonization percentage in both seasons.

Keywords:: root colonization, spore number, Sultan yaghoub, soil phosphorus, mycorrhizal fungi

P656: **Isolation and Identification of xanthomonas species from citrus and investigation of xanthan gum product**

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Background and Aim: Xanthan gum is an extra-cellular Hetero-polysaccharide, which has been synthesized by Xanthomonas campestris and some other types of Xanthomonas. This gum today have been used very much in industries such as food industries,pharmaceutics, publishing, painting, paste,oil,agriculture and etc.the aim of the this stady was to Isolation and Identification of endemic Xanthomonas Species from Citrous and Study Production of Xanthan Gum.

Methods: samples were collected from citrus canker of jiroft iran and plate on yeast extract Dextrose caco3(YDC).next the isolated capable of xanthon gum production on YDC were selected. For the production of Xanthan gum from isolates, following production media.isolate was identified on morphological observation an 16srRNA analysis.

Results: 2 different isolates designated as Xanthomonas sp. The Xanthan production was obtained in the rangs of 0.081-1g/100ml.

Conclusion: in this study the localized strains was used for the xanthan gum production.

Keywords: xanthan gum.YDC, Xanthomonas campestris

P657: **Isolation and Identification of soil origin Actinomycetes and evaluation of their antimicrobial pigments**

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Background and Aim: Actinomycetes are the soil microbiota and they could produce a wide variety of bioactive compounds. Nowadays, frequency of occurrence of antibiotic-resistant bacteria is increasing, hence a new source of remedy could be considered as a main field of investigation. The major purpose of this study was isolation of Actinomycetes from soil samples and the evaluation of antimicrobial pigments production.

Methods: In general, 25 strains of Actinomycetes were isolated from 80 soil samples and evaluated for production of antimicrobial pigments. To perform the test, one gram of soil sample was serially diluted 10 -1-10-7 using sterile distilled water and plated on nutrient agar, starch casein agar, ISP Medium No.2.Then the isolates transferred into nutrient broth media and incubated in a shaker incubator (150 rpm)for 48h for production of pigments. The pigments extracted by ethylacetate and then antimicrobial property of each pigment was assessed by Well Diffusion Agar method (WDA) against pathogen microorganisms viz., Escherichia coli, Pseudomonas aeruginosa, Bacillus cereus, Staphylococcus aureus,Shigella,Citrobacter,Klebsiella sp,Entrobacter aeroginusa and , Aspergillus sp, Candida albicans .Finally pigments producing Actinomycetes with antimicrobial characters were identified by Api kits(coryne).

Results: The obtained results indicated that six strains of Actinomycetes isolates could produce antimicrobial pigments. The crude pigments showed activity against pathogenic bacteria Bacillus cereus and Staphylococcus aureus, Citrobacter, Entrobacter aeroginusa. The crude pigments showed the maximum zone of inhibition against Bacillus cereus and Citrobacter. However, Aspergillus sp. and Candida albicans were resistant to Actinomycetes pigments.

Conclusion: The present study opined that some strains of Actinomedycetes produce pigments with antibacterial property.therefore,these compounds could be considered a new source of remedy.

Keywords: Actinomycetes, Antimicrobial pigments, Soil

P658: **Identification and comparative study of the growth of cyanobacteria from kharg oil polluted region**

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Background and Aim: Kharg island (Booshehr province) is located in Persian Gulf in which one of the most important terminals of oil export is located there. In this research, identification and growth study of the cyanobacterial (microalgal) flora of this island carried out for the first time.

Methods: Sampling was done in Jan. 2012 from various parts of soil and water of Kharg island. After transferring the specimens to lab, the microalgae were isolated by agar plate method using BG11, BG110, N8 and F/2 culture media. After continuous subcultures and gaining the pure colonies from specimens, their semi permant slides prepared by monte-glycerine method and morphologically identified by identification keys such as Prescotte (1962), Desikachary (1959) and John (2003). Among these specimens, the ones that had the most density in the surface of the plates were considered as the dominant species and their growth rate and curves were studied spectrophotometrically.

Results: The following species were identified as: Phormidium rubrolerricola, Phormidium angustissimum, Synechococcus elangatus, Fischerella musicola, Schizothrix vaginata and Nostoc muscorum. The highest growth rate was belonged to Phormidium angustissimum (μ= 0.3).

Conclusion: According to the results, these specimens can introduce as candidates for oil pollution phytoremediation.

Keywords: cyanobacteria, growth rate, identification, Kharg, morphology, phytoremediation.

P659: **Comparison of three phenotypic methods for Isolation and Identification of Nocardia spp. from soil**

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Background and Aim: The aerobic Actinomycetes are a large group of soil-indwelling bacteria that are distributed in worldwide. These gram-positive bacteria are most commonly associated with opportunistic infections in both immunocompromised and immunocompetent hosts. The aim of present study is comparison of three phenotypic methods for Isolation and Identification of Nocardia spp. from soil.

Methods: A total 70 soil samples were collected from different locations in Isfahan's suburb. In this study, three phenotypic methods were compared for isolation of Nocardia: 1- Paraffin Baiting method 2- paraffin coated slides and 3- Slip-buried-method. Isolation methods: 1. Paraffin baiting technique: Soil samples were added to carbon free broth then pre-heated at 550 C in water bath. The supernatants were transferred into tubes. A paraffin waxed glass rod was soaked in each tube and carbon-free broth added .Tubes were incubated at 350 C and 420 C for 3 weeks. Then, the colonies were streaked on brain-heart infusion (BHI)-blood agar with cycloheximide and chloramphenicol. 2. Paraffin coated slides: slides were coated with melted paraffin and 1M NH4CL. Immediately, the slids were put under the soil and then incubated. afterward, the colonies were streaked on SDA medium with cycloheximide. 3. Slip-buried-method: Soil samples were added to normal saline. Tubes were shaken and the suspensions were incubated. Supernatant solution was transferred to another sterile tube. The Streptomycin/chloramphenicol solution was added to the supernatant then incubated. Samples were cultured on BHI-blood agar. Cycloheximide and kanamycin also added. They were incubated at 370 C for 2 weeks.

Results:: First and second phenotypic methods were applied to soil samples but no results attained. Therefore, third method of phenotypic was tested. Utilizing the third technique, 14 out of 70 soil samples (20%) were positive for Nocardia spp and the best result in this study was Slip-buried-method. After culture on BHI and SDA mediums with antibiotics by third method, the mediums kept for two weeks incubation at 370 C. After this time, colonies were observed in chalky form and wrinkled the colonies stained with Kinyoun Carbolfuchsin for microscopic observation with seeing of these colonies in color of red, orange, yellow, and from white to cream, within this period and observation of partially acid-fast organisms in reddish color to purpule filaments.

Conclusion:: In the present study as mentioned, three techniques surveyed for isolation of Nocardia, results showed that the third technique (Slip-buried-method) was better than other techniques. In third technique, BHI agar medium including two antibiotics (kanamycine and cycloheximide) were used and it showed that the growth of Nocardia and Actinomadura were provoked by kanamycin. In this study, Nocardia frequency in soil was 20%. Isolation of Nocardia spp. from different zones of Isfahan, and the effect of environmental factors such as soil pH and different climates were assayed Nocardia diversity in soil.

Keywords: Nocardia spp., phenotypic methods, soil, Isfahan

P660: **Isolation of riboflavin producer yeast from dairy products and optimization of vitamin production**

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Background and Aim: Riboflavin that commonly known as vitamin B 2 , is an important B vitamins for maintaining human health and owing to its particular physiological role, it has been widely used in the fields of feed and food additives and pharmaceuticals. Certain microorganisms have the potential for natural production of vitamins and some fungi, bacteria and yeasts can produce riboflavin. So the main purpose of current study is isolation the yeasts which are capable of producing riboflavin and then investigation of the effect of different sources of carbon and nitrogen on riboflavin production.

Methods: In current research, yeasts were isolated from various dairy products and all colonies were examined for riboflavin production. Then spectrophotometer, TLC and HPLC methods were used to identify and analysis this vitamin in the production medium. After determination of the best producer between all of strains with these methods, In order to determine the best sources for production, different carbon and nitrogen sources were evaluated. Sucrose, lactose, carboxy methyl cellulose and starch as carbon sources, and peptone, trypton, yeast extract, meat extract and potassium nitrate as nitrogen sources were tested. After selection the best sources, different concentration were added to production medium.

Results: First of all, isolation of yeasts from dairy products was done and then spectrophotometer, thin layer chromatography and high performance liquid chromatography was used to identify and analysis this vitamin in the production medium that obtained at the end of incubation period. After determination of yeast producer between all of strains with these methods, identification of the best producer was carried out by morphological, biochemical and molecular techniques. Also results showed that yeast extract 0.7% and sucrose 2% were the best nitrogen and carbon sources. Potassium nitrate and starch had no effect on riboflavin production.

Conclusion: Recently the riboflavin production is shifting from chemical synthesis to microbial production due to advances in metabolic and genetic engineering. In present study a simple method was used to isolate yeasts can produce vitamin. Also it is important to know there are many factors that affect riboflavin biosynthesis, which varies in each microorganism. The conventional method that has been used for optimization of riboflavin production is the one-factor-at-a-time approach in which a single factor is varied while fixing all others at a specific level, so in our study “one-at-a-time” experiment was done to work on the effect of selected carbon and nitrogen sources on production.

Keywords: Yeast, isolation, optimization, carbon and nitrogen sources

P661: **Screening of water and sediment samples for isolation of Sulfur reducing bacteria (SRB)**

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Background and Aim: Sulfur reducing bacteria are a diverse group united by their anaerobic nature and the ability to use elemental sulfur or sulfate and other oxidized sulfur compounds as electron acceptors during anaerobic respiration. Because significant amounts of sulfate are present in almost all aquatic and terrestrial habitats, Sulfur reducing bacteria are widespread. So the present investigation aimed at the isolation and identification of SRB from water and sediment samples of Tembi river in Khuzestan province.

Methods: Water and sediment samples were collected from Tembi river and then isolation of bacteria was done on synthetic medium containing different concentration of NaCl. Whereas these bacteria are unculturable on plate, medium in this research was prepared in vials. After purification with serial cultures, molecular identification was done.

Results: Most probable number counting of sulfur reducing bacteria on a defined NaCl concentration gradient showed that there is a very different microbial community in sediment and water samples of the upstream and downstream of the Tembi river. these bacteria are enriched in the ground water and also in the vicinity of river and their sulfate reduction resulted in H2S production. High H2S concentration resulted in Tembi water toxification.

Conclusion: There are seven sulfur and sulfide springs at Golgir region that introduce in to the Tembi river and result in a detectable hydrochemical and microbioecological changes as our results showed. Such specific microbioecological properties together with a long salinity gradient introduced the Tembi river as a specific ecosystem for biodiversity and geomicrobiology studies.

Keywords: Isolation, Sulfur reducing bacteria, Tembi river, NaCl Concentration

P662: **Isolation of Sulfur oxidizing bacteria (SOB) from sediment samples**

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Background and Aim: Microorganisms that are capable of oxidizing reduced inorganic sulfur compounds are known as sulfur oxidizing bacteria (SOB). Most of the known sulfur-oxidizing bacteria belonging to genera Thiobacillus, Thiothrix, Beggiatoa, Thiomicrospira and Achromatium . These bacteri have the ability to oxidize reduced sulfur compounds and gain energy from these processes. The end product of oxidative activity is the production of sulfate which is extremely stable towards further chemical activity in nature. The main purpose of this research was isolation of SOB from sediment samples.

Methods: In all cases 10 g of the sediment samples had be suspended in 90 ml of the autoclaved water samples of the springs. Flasks should be tightly capped with robber cap or like it and incubated at 28-30oC. Samples that showed turbidity in the medium was chosen for purification. So cultures should be serially diluted using the same sterilized water sample and enrichment media for 5times. In order to, SOB usually developed a thin layer on the surface of medium, after observation of this layer, molecular identification was done.

Results: In our research, we observed presence of SOB in three samples of seven sediment samples, so found that hydrochemical and ecological properties have the effect on the microbial biodiversity.

Conclusion: The sulfur-oxidizing bacteria can be isolated from acid, neutral or alkaline environments, form cold, moderate or hot habitats, as well as from low to highly saline waters and soils. So in this study sulfur-oxidizer was isolated from sediment samples of Tembi river in Khuzestan province.

Keywords: Isolation, SOB, Tembi river, sediment samples

P663: **Pseudomonas panipatensis and its role on growth of capsicum annuum**

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Background and Aim: Pseudomonas is a gram negative, motile, catalase and oxidize bacterium belong to the family of pseudomonaceae. Among the species the panipatensis is ubiquitous in nature due to their metabolic versatility and capability to enhance growth of plants. In deed some species of Pseudomonas and Rhizobacteria induce plants growth, and has a very significant role in bud growth increasing, tissues nutrient concept, stem weight & height, chlorophyll content, increasing of node formation and early blooming. Hence the aim of this study is isolation of this species from soil and evaluation of its metabolite on growth of Capsicum annuum.

Methods: In the present study 37 soil samples has been collected from around Marvdasht region in Fars Province. According to serial dilution & cultivation on nutrient agar and cetrimide agar, the colonies with different morphology, color & foundation were identified using biochemical & molecular methods. Then 24 pots were provided & filled with sterile soil. In 12 pots the pepper seeds inoculated with isolated pseudomonas and the rest were remained as test groups. After 80 days the pots were evaluated. Furthermore, the isolated bacteria from soil of pots were evaluated for indole acetic acid & ammonia production.

Results: The results obtained from this study indicated that the pots which inoculated with isolated pseudomonas from soil showed more growth regarding to the number of leaves, bush height, stem diagonal and root lengths than the tests. Furthermore the results illustrated that the isolated bacteria were belong to genus Pseudomonas which according to molecular identification was Pseudomonas panipatensis strain ESP1 (NR_044209.1). In addition the isolates were able to produce indole acetic acid and this compound had effect on growth of capsicum annuum while the isolate has not ability to produce ammonia.

Conclusion: According to obtained data from this study it could be concluded that Pseudomonas panipatensis strain ESP1 has a role in plant growth & there is possibility to use the isolates in further studies.

Keywords: Pseudomonas panipatensis, capsicum annus, IAA

P664: **Biodesulfurization of Dibenzothiophene using cell- free extract of Rhodococcus erythropolis R1**

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Background and Aim: the Combustion of Hydrodesulfurization-refractory organic sulfur compounds such as Dibenzothiophene (DBT) in fossil fuels emits sulfur oxides, which can cause air pollution and acid rain. Several types of bacteria were able to remove selectively the sulfur from DBT without breaking the carbon ring by 4S pathway. Biodesulfurization (BDS) is a multi-enzymatic reaction . Enzymatic methods generally have low energy requirements, are easy to control, and no bacteria separation is subsequently required, thus, enzymes can play an important role in the development of biotechnological processes with potential application in polluting industries.

Methods: The enzymatic desulfurization of dibenzothiophene (DBT) to 2-hydroxybiphenyl (2-HBP) was detected in cell extracts of Rhodococcus erythropolis R1 grown on DBT as a sole source of sulfur. In this study, the reaction conditions (pH, temperature and reaction time) were optimized in a liquid medium by a series of single factor experiments. The reaction mixture (final volume, 2.5ml) contained 100mM potassium phosphate buffer, 0.3mM DBT and the cell free extract. The enzymes activity was assayed by HPLC analysis for measurement the amounts of remained DBT.

Results: When the enzyme activity was measured at various pH and temperatures,the optimum situations were found at 30°C , pH 7.4.The maximum activity was observed at 4 hour incubation.

Conclusion: BDS is a multi-enzymatic reaction, and cofactor regeneration is needed. In the purified enzyme, NADH was absolutely required for the activity Whereas cell extract containing this substance. In the present study, we found that unlike whole cells, the presence of sulfate in DBT desulfurization by cell free extract had no inhibition effect on the desulfurization enzymes activity.

Keywords: Biodesulfurization, Dibenzothiophene, cell- free extract, Rhodococcus erythropolis R1

P665: **The frequency of Mycobacterium lentiflavum as a rare potentially pathogenic organism in Isfahan hospital water systems.**

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Background and Aim: In recent years, non-tuberculous mycobacteria (NTM) have emerged as important cause of opportunistic nosocomial infections. Among environmental samples, water is a powerful environmental determinant of health and has a major role for transmission of opportunistic pathogens to human especially to immunocompromised patients in hospitals. Mycobacterium lentiflavum is a potentially pathogenic organism that causes a wide variety of nosocomial infections. In current study we carried out an investigation for detection of M. lentiflavum in Isfahan hospital water supplies with the aim of casting light on possible reservoir of this rare organism in the environment.

Methods: A total of 140 water samples were collected from hospital water resources in Isfahan Iran. They were subjected to filtration and decontamination by using 0.05% CPC (cetylpiridinium chloride) and sub-cultured on Lowenstein Jensen medium and incubated at various temperatures for two months. The isolates with characteristics of mycobacteria were identified at the species level by a set of biochemical and molecular tests including 16srDNA gene sequencing.

Results: Out of 140 water samples collected from hospitals in Isfahan province 23(16.5%) Mycobacterium lentiflavum isolates were identified.

Conclusion: Our data showed that M. lentiflavum is a rather ubiquitous organism in Isfahan hospital water and might be involved in neglected nosocomial infection in patients particulary in those with immunocompromised conditions.

Keywords: Mycobacterium lentiflavum, Hospital water, 16srDNA

P666: **Isolation and Identification Of New Species of Soil Chitinolytic Bacilli in North Of Iran**

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Background and Aim: Chitin is a linear polymer of N-acetyl glucosamine units which, is the most abundant biopolymer after Cellulose in the nature. In recent years due to their extensive applications, there has been much interest in Chitinase especially as a biological control against fungi.

Methods: In this study, isolation of chitinase-producing Bacillus was done by collecting 40 samples of soil from four geographical regions in Gorgan and the decomposition feature of Chitin was evaluated by examination of transparent halo in plates containing Colloidal chitin agar as the only source of certain carbon and nitrogen. Identification of selected strains was carried out based on biochemical and molecular characteristics.

Results: Totally 9 colonies of chitinase positive Bacillus on colloidal chitin agar plates were isolated between these colonies R2 and R7 had the most and the least (max and min) chitinase activity, respectively. these strains were sent to Korean Macrogen Company for precise identification after DNA extraction. After comparing 16S rRNA sequence, of these isolates with known bacteria, and considering their morphological, physiological and genetic differences, related data was sent to NCBI and after examining they were recorded as new Bacilli.

Conclusion: Terricolous chitinoliyic Bacilli can be studied for industrial production of chitinase and their anti-fungal effect on pathogenic fungi can be examined.

Keywords: New Species, Bacillus, chitinase, Soil, Iran

P667: **Detection of coliform bacteria and Escherichia coli in the southwest the Caspian Sea by Using the Polymerase Chain Reaction and Gene Probes for Uid**

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Background and Aim: Considering that transmission of many diseases through the water Result in contamination of water sources, types of microorganisms with feces human and animals, Therefore identifying the various sources of fecal contamination of drinking water is very important in controlling these diseases.

Methods: The aim of this study was to determine the extent of fecal contamination of water sources in four different regions of the southwestern Caspian Sea, by Using the polymerase chain reaction and Gene Probes LacZ (β- D galactosidase gene) and UidA (β- D glucuronidase gene) has been used to detected total coliforms and E. coli, respectively.

Results: PCR analysis of used Gene Probes LacZ of 60 samples, 55 cases (91.6%) of total coliform and PCR product was 264bp and using gene probes UidA only 44 cases (73.3%) showed positive reaction and PCR product was 147bp.

Conclusion: Consequently, The method could be an effective epidemiological tool to pin point the source to contain outbreaks of waterborne disease episodes.

Keywords: water resources, Coliforms, polymerase chain reaction, beta-galactosidase, beta-Glucuronidase.

P668: **Evaluation of antimicrobial effects in Tonekabon soils Streptomyceses against Escherichia coli (O157: H7) and MRSA**

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Background and Aim: Streptomyces are prokaryotic microorganism that have the power to produce different metabolites. Secondary metabolites in Streptomyces have many Usage in Pharmaceutical Industry. These bacteria produce different Substances such as: antibiotics, enzymes and vitamins that they are important for human health. The aim of this study was to evaluate antibacterial effects of secondary metabolites in Streptomyces isolated from Tonekabon soils against standard and pathogenic strains Escherichia coli (O157H7) and Methicillin-resistant Staphylococcus aureus (MRSA) that the infection caused by this two pathogens is increasing in many countries.

Methods: Soil samples were collected in depth of 10-15 centimeter from different parts off Tonekabon city. Streptomyces were identified to genus according to regular microscopic, macroscopic and biochemical methods. then, their antagonistic Characterization against Escherichia coli (O157H7) and Methicillin-resistant Staphylococcus aureus (MRSA) was evaluated by cross streak method. In the next step, microbial extraction was taken from Streptomyces that had suitable antagonistic effects and then, antimicrobial metabolites were purified by Ethyl acetate and the antimicrobial characteristic of purified extraction was evaluated by disk diffusion and well diffusion methods. Then Superior Streptomyces in production of antibacterial metabolites were identified by polymerase chain reaction and sequencing of PCR product according to16SrRNA sequence.

Results: In this study 10 superior Streptomyces were identified according to microscopic, macroscopic and biochemical tests, these bacteria were highly active against Escherichia coli (O157H7) and Methicillin-resistant Staphylococcus aureus (MRSA).one of the isolated bacteria was identified Streptomyces.sp by morphological and 16S rRNA sequence analysis.

Conclusion: Today because of antibiotic resistance pathogens development, demands for novel antibiotics is increasing. According to the results in this study it has identified that Tonekabons soiles have good potential to find antimicrobial productive Streptomyces.it seems that this study is hopeful to cure developing infections, and there are few studies about antibacterial productive Streptomyces in soil and according to ecological diversity in Iran, it is possible to find new antibiotic productive species in other cities of country.

Keywords: Streptomyces, secondary metabolites, antagonism, 16S rRNA , E.coli (O157H7) , Methicillin-Resistant Staphylococcus aureus, antibiotic, ethyl acetate

P669: **Identification and isolation of Non-tuberculous mycobacteria from water sources in Iran**

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Background and Aim: Nontuberculous mycobacteria (NTM) differ from tuberculous mycobacteria as most of them are ubiquitous and saprophytic. A few are considered to have the potential to infect humans. The immunological status of a person determines the advance of disease. On the other hand among environmental factors, water plays the main roles as a resource of contamination in transferring this group of microorganisms to human. The aim of this study is to identify and isolate NTM in the different region of Iran surface water.

Methods: A total of 104 water samples were collected from different parts of Tehran city, North and south of Iran. Cetyl pyridinium chloride (cpc) 0.1% was used to decontaminate the water samples. After enrichment using filtration, all samples were incubated on Lowenstein-Jensen (LJ) medium at temperatures of 30 and 37° C. Classical culture, biochemical and enzymatic methods are described for the identification of mycobacteria and the last value was determined.

Results: From the total of 104 water samples, 37(35.5%) mycobacteria have been isolated. 30% belong to north of Iran, 47% south of Iran and 33% from Tehran city water sources.

Conclusion: This study indicates that notable percentage of water sources are contaminated with mycobacterium species. The exposure of immuno-compromised individuals to these sources can have crucial consequences. Interestingly the prevalence of mycobacterium species differs in diverse geographical areas.

Keywords: Non-tuberculous mycobacteria, water, Different regions

P670: **Evaluate and compare the antimicrobial properties of Streptomyces isolated from the northern and southern seashores of Iran**

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Background and Aim: Serious infections caused by bacteria that have become resistant to commonly used antibiotics has become a major global healthcare problem in the 21st century. Streptomyces, with the potency to produce secondary metabolites, have medical and pharmaceutical applies. The aim of this study was to isolate native marine Streptomyces by the purpose of estimating their potency in producing secondary metabolites with inhibitory effects against antibiotic resistant bacteria.

Methods: ISP2 specific culture medium was used to isolate native marine Streptomyces from Qheshm Island, Caspian Sea and Valesht Lake. Colonies with the characteristics of Streptomyces were transferred to fermentative Starch and ISP2 broth media to produce secondary metabolites. Antimicrobial products extracted and then purified by the use of ethyl acetate. The antimicrobial effects of secondary metabolites were assayed by well diffusion method. Six bacteria, including three Gram positive(Staphylococcus aureus, Micrococcus luteus, Bacillus cereus) and three Gram negative (Pseudomonos aerugenosa, Klebsiella pneumonia, E.cli)and MRSA were used to determine the antimicrobial activity of the isolated Streptomyces strains.

Results: Among 36 isolates tested, 20 species were found to be antibacterial metabolite producer against seven test microorganisms including multiple antibiotic resistant bacteria. All of the isolates were highly active with an inhibition zone more than 20 mm in diameter against MRSA. Most of the isolates inhibited growth of the Gram positive bacteria tested. 15 isolates produced antibacterial substances against both Gram negative and Gram positive bacteria. Eight isolates from Qheshm island showed antibacterial activity on Gram negative bacteria tested. Especially, one of the isolates from Qheshm island inhibited the growth of all of the tested microorganisms with >22 mm inhibition zones.

Conclusion: In this work, we have shown that a total of 20 different Streptomycetes isolates associated with Water have the ability to produce antimicrobial compounds against microorganisms, especially multiple antibiotic resistant Gram positive and Gram negative bacteria. Further investigations are needed in order to further determine the active metabolites of these isolates.

Keywords: ISP2, inhibition zones, Streptomyces

P671: **Taxonomic Classification of Three Newly Isolated Archaeal Strains from Aran-Bidgol Hypersalin Lake**

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Background and Aim: Aran-Bidgol hypersaline lake which is located in the central part of Iran, has been explored with great extreme haloarchaeal diversity in recent years. Extreme halophilic Archaea are widely distributed in hypersaline habitats such as lakes and salterns.

Methods: During the course of biodiversity studies in Aran-Bidgol lake, we isolated several extreme halophilic Archaea some of which could represent new taxa. Amongst these strains we chose strains IC38, IC35, and Hg15 for further characterization and each of these strains showed 16S rRNA gene sequence similarity with Halovivax asiaticus EJ-46T (96.4%), Halovivax asiaticus EJ-46T (95.4%) and Natronomonas moolapensis 8.811T (99.5%) respectively. According to minimal standards for characterization of haloarchaeal strains, we also performed some morphological, biochemical and physiological tests for aforementioned strains.

Results: Cells of strains Hg15 and IC38 were pleomorphic and strain IC35 was bacillus. These strains were catalase positive with optimum NaCl between 20 to 30 % (w/v), optimum pH range between 7 to 7.5 and optimum temperature C for growth. All of the strains were unable to°range between 35 to 40 hydrolyze starch, DNA, tween 40 and tween 60. They were unable to produce H2S, all were MR positive and VP negative. IC38 showed different results for acid production from, galactose, sucrose, starch, fructose, lactose, ribose, mannitol, glycerol and maltose glucose, opposite to all negative results for other two strains. Different results also have been seen in using these sugars as sole source of carbon in their metabolism.

Conclusion: All of these three strains were resistant to amikacin, amoxyclave, carbenicillin, cephoxitin, streptomycin, tobramycin, nalidixic Acid, cephalotin and penicillin while all of them showed susceptibility to some other antibiotics such as rifampicin and Baciteracin. According to obtained data these strains have the potential to be proposed as new native taxa from this extraordinary environment. For this regard, we need to complete the polyphasic scheme for classification of these strains as the member of new taxa in species or genus level.

Keywords: Aran-Bidgol Hypersaline Lake, Haloarchaeal, polyphasic taxonomy

P672: **A study on DON (Deoxynivalenol) production in culture medium by Aspergillus spp. isolates from different geographic areas of northern Iran**

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Background and Aim: According to the increased fungi contaminations and related damages, microbiologist's incentive in considering the fungal contaminations in human habitats had increased. Some of fungi cause disease through production of toxins in animals and thus in humans. Since these toxins are not easily distinguishable, then it is crucial to study their characteristics. Aspergillus are among the most important toxigenic fungi which are found abundantly in northern Iran habitats where is one of most important habitats of Iran and which is the main source for many feed and food stuffs in the state thus we aimed to study on DON production characteristics in culture medium by 24 Aspergillus spp. isolated from different geographic areas of northern Iran isolates collection.

Methods: Samples were collected from Northern Alborz and southern Caspian Sea agricultural plants cultivation areas and processing centers. The mould samples were then isolated and identified based on CBS environmental sampling rules and ICPA diagnostics standards then were cultured to stimulate the toxin production till the targeted toxin to be measured at culturing substrate and fungi biomass. Afterward, they were exposed to extraction and then existing DON size was measured by ELISA technique.

Results: Amongst obtained species, in the West of Gilan; A. parasiticus (65.478 ppb), the East of Gilan; A. melleus (82.581 ppb) and the West of Mazandaran; A. sojae (95.435 ppb) had the highest DON toxin production mean.

Conclusion: According to prepared results, the West of Mazandaran have the highest DON toxin production followed by the East of Gilan and the West of Gilan. Value of measured toxin by ELISA showed that all of mentioned species had toxin production mean less than appointmented permissive limit by FDA for feed while they had mean more than appointmented permissive limit by ECS for infants food by which had mean less than appointmented permissive limit by FAO for adults food.

Keywords: DON, Aspergillus, Species, Geographic areas, Iran.

P673: **The effect of oil pollutants on physiological responses of unicellular alga Chlorella sp.**

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Background and Aim: Oil as an organic compound, not only produces energy but also is the basic element of today's man essentials. But unfortunately it causes widespread environmental pollutants during different steps of oil hydrocarbons extract, transfer and refining. In this study the effect of crude Oil, Toluene and Hexadecane as oil pollutants were evaluated on some of physiological responses of unicellular alga Chlorella sp. such as the rate of photosynthetic pigments, Chlorophyll and photosynthesis.

Methods: For increasing the algal biomass, after purification through plate agar method, the colonies were transferred to culture medium N8 .When the algal sample reached to logarithmic phase; a carbonless culture medium was prepared and treated with crude Oil, Toluene and Hexadecane with 1% concentration separately. Chlorophyll rates were calculated through optical density and photosynthesis were evaluated with Oxyview instrument.

Results: The results showed that the most rates of chlorophyll and photosynthesis belonged to control and Toluene reduced the biomass extremely.The algal growth rate has showed significant enhancement in crude Oil and Hexadecane, although Toluene was so much poison for this alga.

Conclusion: In conclusion, the results implied that crude Oil and Hexadecane had the most positive effects on physiological responses of Chlorella sp.

Keywords: Chlorella, Chlorophyll, Hexadecane, Photosynthesis, Photosynthesis pigments, Toluene

P674: **The variation of physiological responses in cyanobacterium Schizothrix vaginata ISC108 under oil compounds treatment**

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Background and Aim: Today's man encounters many concerns; one of them is environmental pollutants derived from population growth, industriation and technology development. These pollutants emerge from different sources and one of them is oil pollutants. In this study, the variation of physiological responses in Schizothrix vaginata ISC108 under oil treatment such as Crude Oil, Hexadecane and Toluene were analyzed.

Methods: This cyanobacterium was transferred to BG11 liquid culture after purification in solid culture medium. Afterward, Schizothrix vaginata ISC108 in logarithmic phase was treated in a carbonless BG11 medium enriched with 1% crude Oil, Hexadecane and Toluene. The rate of photosynthetic pigments such as chlorophyll and phycobiliproteins were evaluated spectophotometrically and photosynthesis was calculated by means Oxyview.

Results: The results expressed that in comparative by control, the growth rate under Hexadecane and crude Oil treatment was substantial and in Toluene treatment had a few reduction whereas chlorophyll, photosynthesis and phycobiliproteins rates in control were more than treatments.

Conclusion: In conclusion,Schizothrix vaginata is one of the cyanobacteria persists in oil pollutants especially Toluene.

Keywords: Chlorophyll, Hexadecane, Oxyview, Phycobiliproteins, Toluene

P675: **Isolation of phenanthrene degrading bacteria from oil contaminated soil**

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Background and Aim: Polycyclic aromatic hydrocarbons (PAHs) are omnipresent compounds found worldwide in soils and sediments. Phenantherene (one of the toxic PAHs compounds) with three fused benzene rings is one of the major soil pollutants and due to its hydrophobicity, tends to interact with particulate matter, such as clays and humic acids that reduces their removal efficiency. There are several methods commonly used for the soil remediation including dredging and burning, that can be costly and lead to further damage to the environment. PAHs are naturally degraded by microorganisms and biological treatment is believed to be more effective, besides noninvasive and relatively cost-effective.

Methods: This study was designed to isolate phenanthrene degrading bacteria from oil-contaminated soil. Soil samples were collected from oil contaminated soil near Tehran refinery. Bacterial enrichment cultures were set up in cotton-plugged erlenmeyer flasks containing 50 ml of mineral salt medium using phenanthrene as the sole source of carbon. By a multi- step enrichment and screening technique a bacterium was isolated and by morphological characteristics, biochemical tests and phylogenetic analysis method using 16S rRNA sequencing, it was identified as the Mesorhizobium Sp.

Results: . Our results showed that selected strain grow well in pH range of 4-9 and temperature range of 30- 40 °C with optimal growth at 35°C, had an acceptable growth at 5% NaCl and was able to degrade 70 percent of the 300 ppm phenanthrene within 24 days.

Conclusion: Subsequent genetic studies of its degradation pathways may lead to the discovery of novel genes involved and further investigation is required to confirm the abilities of the strain.

Keywords: Phenanthrene, Mesorhizobium, Isolation, contaminated soil

P676: **Mercury resistance in Kocuria sp. ASB 107, a gamma -tolerant bacterium isolated from Ab-e-Siah radioactive spring in north of Iran**

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Background and Aim: Kocuria sp. ASB 107 is a psychrotrophic radio-resistant bacterium isolated from Ab-e-Siah radioactive spring in Ramsar- Iran. The strain has moderate resistance to gamma-irradiation with D10 value of 2 kGy. Since mercury is most prevalent heavy-metal contaminants in radioactive wastes, we studied mercury resistance in Kocuria sp. ASB 107 with comparison of mercury resistance in Deinococcus radiodurans.

Methods: Mercury resistance was determined in the presence of different concentrations (1 - 30 ppm) of HgCl2 in LB broth and TGY broth for Kocuria sp. ASB 107 and D. radiodurans, respectively. Growth of the bacteria was measured spectrophotometrically (A600nm) after incubation at 30°C and 150 rpm. To investigate of adaptation to mercury, Kocuria sp. ASB 107 was grown by serial sub-cultivation on LB broth, with increasing concentrations of HgCl2.

Results: Kocuria sp. ASB 107 was capable to grow in LB broth with 15 ppm HgCl2, while D. radiudurans did not grow in the presence of 5 ppm HgCl2. After the adaptation procedure, Kocuria sp. ASB 107 could grow up to 30 ppm HgCl2.

Conclusion: These characteristics support prospective development of the bacterium for bioremediation of radioactive waste sites that contain hazardous mixtures of radio-nuclides and heavy metals.

Keywords: Mercury, resistance, Kocuria ASB 107, adaptation

P677: **Isolation and identification of Deltamethrin insecticide degrading bacteria from agriculture soil in Marvdasht**

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Background and Aim: Aims: Deltamethrin is one of the synthetic pyrethroid in secticide . it is widly used in agriculture and forestry to control pest. Concentrations of this insecticide found in surface waters and soils . this substance shows high toxicity to fish and animals. Microorganisms are highly effecting in transforming organic pollutants and modifying there structure and toxic properties . they can completely mineralize organic compounds to nonorganic products

Methods: Material methods: from place of soil was don sampling . samples injectioned to MSM 30 cc deltamethrin and incubated in 28 degry . after identifying the bacteria . the guantity of degrading deltamethrin measured by GC.

Results: Results: after 15 days incubation , deltemethrin was degraded by gram positive bacteria 23% and gram negative bacteria 69% . the level of deltamethrin biodegradation in mixed culture of gram positive and gram negative bacteria was higher than that in homogenous cultures

Conclusion: Conclusion: the result of the research by GC showed that Pseudomonas sp. And kelebsilla Isolated was the most effective in reducing the concentration of this insecticide . growth kinetic of pseudomonas was studied

Keywords: key word: biodegradation, gas chromatography, deltamethrin

P678: **Isolation and Identification of Pseudomonas Bacteria Radio-resistant from hot springs of Ramsar**

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Background and Aim: One of the greatest human achievements of the twentieth century is discovery of radioactivity and nuclear interactions and finding out it`s properties of radiation. Of Course these discoveries has been studied and researched on effects of radiation on living organisms and the nature of environmental resources . One of the most important consequences of working with radioactive, production of gamma rays during the breakdown of a radioactive nucleus. Ground radiation source with radioactive elements that have half-lives find in hot springs that were seen mostly in the Earth's crust in volcanic areas. Ramsar areas is one of the areas with high natural radiation (HLNRA) . This may be due to exposure to high levels of radium in hot springs, rocks and soils attributed Ramsar area. In order to clean up the area of radioactive materials, many methods have been proposed. One such method is the use of bacteria that have the ability to survive in gamma ray irradiation.

Methods: Sampling was done first hot spring hotel Ramsar. For the isolation of bacteria from the culture mediums (chocolate agar, eosin methylene blue agar, MacCanky agar, Mueller Hinton agar) were used for continuous cultivation of a culture, and single clones were obtained. Then the balk culture of selected bacteria, and control bacteria (E.coli, with no resistance), were irradiated with gamma rays in Atomic Energy Organization of Tehran. the survived Strains that was assessed by spectrophotometer reported as a resistant bacterium from hot springs hotel`s of Ramsar. To further investigate the biochemical characteristics of bacteria, we had several tests including Oxidase test, Catalase test, analysis on urea.

Results: According to a survey carried out by bacteria isolated from hot springs in Ramsar, it is a strain of Pseudomonas. Urea analysis test: Urease enzyme helps to recognize that Pseudomonas can produce a identify. After inoculation of the medium changes color if the result was positive. Catalase test: Immediate positive reaction with the formation of gas bubbles (oxygen) bacteria was created on clones which is mass markers of Pseudomonas. Oxidase Test: This test is for identify bacteria that have Cytochrome Oxidase activity. Oxidase test in Pseudomonas is positive. Using discs manufactured by MAST Oxidase-positive result was announced with a blue surface.

Conclusion: In water samples, from hot spring of hotel Ramsar, colonies were isolated and then the bacteria that was resistant to gamma ray, isolated. After the selection of bacterial strains that showed the highest resistance to gamma-rays, we identified them through biochemical tests. According to the biochemical result we showed that this special strain that was isolated is pseudomonas and of course the resistance to gamma radiation make it interested point to bioremediation applications.

Keywords: Bioremediation, Radioactive, Pseudomonas

P679: **Survey of a Variety of Environmental Stress Conditions on the Viability, Cell Morphology and Biochemical Characteristics of Listeria monocytogenes ATCC 19114**

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Background and Aim: Listeria monocytogenes is a particular concern for the food industry due to its high case of fatality, widespread distribution, ability to survive a wide variety of food processing conditions, and the severity of illness associated with foods contaminated with this pathogen. Currently, L. monocytogenes is acknowledged as a significant foodborne pathogen which causes a high fatality rate in humans annually.The purpose of this investigation was to examine the viability, morphological and biochemical characteristics of L.monocytogenes ATCC 19114 that endured selected environmental stresses.

Methods: The environmental stresses investigated were acid shock (HCl, pH2.0-6.0), alkaline shock (NaOH, pH 8.0-12.0), ethanol shock (5.0% -25.0% vol/vol), oxidative shock (H2O2, 0.06%-6.0% vol/vol), osmotic shock (NaCl and sucrose, 2.0%-30.0% wt/vol) and heat shock (40-60°C). All shock were applied to cells at exponential phase whereas non-stressed exponential phase cells served as a control and the cells were allowed to grow for 24 h. For evaluating the viability of L. monocytogenes PTCC1297 after inoculation procedure and exposure of cells to selected stresses used colony count method.Scanning electron microscopy (SEM) was implemented to visualize the surface appearance of bacteria after exposing to stress conditions.

Results: The amount of HCl (pH4), NaOH (pH10), ethanol (15% vol/vol), H2O2 (0.3 %vol/vol), NaCl (14% wt/vol) and heat (50°C) were consideredas lethal dose for L. monocytogenes ATCC 19114. Different degrees of sucrose could not kill L. monocytogenes. Our results showed thatexposure to environmental stresses affected the survival, shape and biochemical characterization of L. monocytogenes ATCC 19114.

Conclusion: It can be concluded that more understating about L. monocytogenes stress response can lead to progress and identify the physiology of microorganisms, development of new vaccine, new treatments for diseases, new methods of food health and identify new antimicrobial agents.

Keywords: Environmental Stress Conditions , Listeria monocytogenes

P680: **Isolation and Identification of culturable bacteria from Badab Sourt travertine spring , Kiasar-Mazandaran Province**

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Background and Aim: Travertine is a form of precipitated carbonates from young fresh spring. Badad sourt spring is travertine spring that located on 36° 21.297′N 53° 51.397′E and 1841 altitude. That is second natural place in Iran, have several springhead that are different in physicochemical conditions such as salinity, pH and color. Two of springhead respectively has 5% salt with pH 7.5 and 0.03% salt with pH 6.8. Because of this diversity Badad sourt spring is suitable place for studying microbial diversity.

Methods: Samples with regarding SOP sampling were collected in October 2012 and stored in 4 °C. After preparation of representative samples, water samples were centrifuged and inoculated to nutrient agar with spread method and then serially diluted (up to 106) for sediment samples. The plates were incubated at 37 °C and counted for 3 weeks. Different colonies grown on media were selected and purified, were subjected for further investigation.

Results: A total 151 strains were recovered and partial identification of strains gained; bacteria had different morphology e.g. cocci, bacilli, curved and polymorph

Conclusion: in water sample strains were 25.8 % Gram- negative and 74.2 % Gram- positive and in sediment sample were 9 % Gram- negative and 91 % Gram- positive and 17 % of total strains were spore-forming bacteria

Keywords: Travertine , Badad sourt springe, culturable bacteria, Isolation, Identification

P681: **Isolation of crude oil-degrading bacteria from the coastal area of Qeshm Island**

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Background and Aim: In the process of fulfilling the energy requirement for today's population, various natural resources have been exploited. But the principal source of energy continues to be petroleum hydrocarbon and hence a global pollutant. The traditional treatment of oily wastewater, such as containment and collection using floating booms, adsorption by natural or synthetic materials, etc., cannot degrade the crude oil thoroughly. Bioremediation is a natural process in which microorganisms utilize certain harmful chemicals including oil spills and change them into non-toxic and innocuous substances such as water and carbon dioxide. The present study was conducted to isolate oil-degrading bacteria from the coastal area of Qeshm Island and to assess oil degrading ability of the isolated strains.

Methods: Surface sediments were collected from different sites in from the coastal area of Qeshm Island. Sediments were transferred to conical flask containing mineral salt medium and 1% Iranian light crude oil. At the end of the enrichment, serial dilutions of bacterial consortium were prepared and spread on nutrient agar plates. Purified strains were identified by biochemical tests. To assess the efficiency of isolated strains in utilizing crude oil, isolates were firstly cultivated in mineral salt medium containing sodium acetate (2%), as the sole source of carbon for 2 days, incubated at 35˚C and 140 rpm. Then after, 2ml of microbial inoculums were passed to the MSM containing 50 ml filtered seawater and 1% Iranian light crude oil, incubated at the same condition and adjusted at pH=7 for a week. At the end of experiment remained crude oil was extracted using n-hexane at pH=2 in each conical flask using separating funnel.

Results: Two oil-degrading bacterial strains (KK1 and KK2) were isolated from enriched consortium. KK1 cells were aerobic, gram positive, spherical-shaped, small convex cream colonies, catalase and oxidase positive, while the KK2 cells were aerobic, gram negative, relatively rod-shaped, raised colorless colonies, catalase and oxidase positive. KK2 had stronger ability to degrade crude oil than KK1.

Conclusion: In the present study, only two oil-degrading bacterial strains were isolated from enriched consortium which was lower than those reported in literature. Nevertheless, our results showed that all isolates were capable of degrading crude oil. The use of native or indigenous bacteria for bioremediation is of great interest as it is often more useful and beneficial than commercial inoculum which could be out-competed by indigenous microorganisms, which means the isolates have a promising application in bioremediation of petrochemical contamination in the local environments. Isolated strains showed moderate biodegradation activity, this could be because of little knowledge about the optimum growth of isolated strains. Several researchers have shown, microbial biodegradation efficiency depend on different factors. In essence, assessment of the optimal conditions would make the bioremediation process more effective.

Keywords: Qeshm Island; biodegradation; Bioremediation

P682: **Prevalence of Escherichia coli specific genes by a novel nanoplex PCR: An applicable genetical method to detect Escherichia coli in water samples**

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Background and Aim: Escherichia coli is used as the indicator of fecal pollution of water. Conventionally, there are some phenotypical methods to detect such microorganisms in water like acid and gas production from lactose, MUG hydrolysis and tryptophan metabolism, however they are all time consuming. In contrast, genotypical methods are much more specific, sensitive and rapid, of course if pre enriched and then subjected to PCR. The aim of this study was to determine the most ubiquitous Escherichia coli specific genes to apply as a genetical marker to speed up the detection time and the sensitivity.

Methods: Three hundred and thirty nine Escherichia coli strains, isolated from different water sources of Alborz province within a 4 year period, were characterized by standard methods based on fast lactose fermentation and IMViC test. All the isolates were kept in glycerol at -70ºC until the final procedure. New specific sets of primers including structural genes like uspA, phoE, lamB, tuf and enzyme coding ones such as gadA/B, tnaA, uidA, cadA and mdh were designed in a novel nanoplex PCR by Allele ID 7.6. Following strict optimization, specific pmoles of each set of primers with an adequate volume of template DNA could characterize nine obvious and sharp bands indicating nine corresponding genes of interest in a single reaction. To exclude any Shigella, we designed a specific primer for putative integrase. However it should be noticed, despite the great DNA homology with Escherichia coli, Shigella (sonnei) is not/or is slow lactose fermenter. Following PCR, amplicons were electrophoresed in a 1.5% agarose gel, visualized by a Gel DocTM XR+ (BIORAD) and analyzed by Image LabTM software.

Results: Following digitizing (0 & 1) the positive genes and dendrogram construction by PAUP 4.0 software, the nanoplex PCR allowed genotyping of the 339 environmental E.coli strains into 8 profiles among which, the members of the first profile made up 87.31% of total. In this survey, the prevalence of the genes were 100%, 100%, 100%, 99.71%, 99.41%, 99.41%, 99.11%, 89.67% and 99.11% for uspA, lamB, tuf, phoE, gadA/B, tnaA, mdh, cadA and uidA, respectively.

Conclusion: Our results showed the most ubiquitous specific genes of Escherichia coli, as well as the other cells, are the structural ones encoding for the structural proteins. These results are highly justifiable because the structural proteins serve as cell components and if absent or deficient, the cells have no chance to survive because of selection pressure. In spite of being specific, the mentioned genes encoding for the enzymes are prevalent but not absolutely ubiquitous. These results are also justifiable because the absence or deficiency of these enzymes is covered by the wealth of genes, metabolic pathways and other molecular processes and so mutants can thrive by applying some other pathways. In conclusion, any of the structural genes in single or multiplex can be a reliable genetical target to trace Escherichia coli in water samples by PCR.

Keywords:: Escherichia coli, nanocaplex PCR, genotyping

P683: **Evaluation of Synechococcus sp. as a candidate for biodiesel production**

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Background and Aim: High price and continued use of petroleum sourced fuels tend the attention of scientists to an alternative sustainable biofeul. The purpose of this paper is to evaluate the potential of cyanobacterium Synechococcus sp. as a candidate for biodiesel production.

Methods: Sampling was performed from soils and waters of Kharg Island in Persian Gulf. After purification, samples were incubated in BG11 culture medium at 25 ºC and constant light of 40 to 60 µE m-2s-1 and pH 7. Identification was carried out morphologically and physiological parameters as growth rate, pigmentation and photosynthetic rate were measured. The total amount of lipid, amino acids and fatty acids profile were measured by FTIR, HPLC and GC.

Results: Allophycocyanin, Phycocyanin and Phycoerythrin of Synechococcus sp. were 12.02, 23.68 and 6.63 ?g mg dw-1, respectively. The amount of amino acid Synechococcus sp. was about 26.15 mM gFw-1.The profile of fatty acids included Myrestic acid (41.44%), Palmitic acid (15.53%), Stearic acid (5.28%), Palmitoleic acid (30.74), Oleic acid (5.13%), Linoleic acid (0.95%).

Conclusion: According to high growth rate and a good pattern of amino acids and fatty acids of Synechococcus sp., this species can be consider as a candidate for biodiesel production.

Keywords: Amino acids, Biodiesel, Cyanobacteria, Fatty acid productivity.

P684: **Physiological and biochemical analysis of cyanobacterium Nostoc sp. for biodiesel production**

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Background and Aim: Cyanobacteria have recently received a lot of attention as a new biomass source for the production of renewable energy. The aim of this paper is to study the growth rate, photosynthetic pigments and fatty acids profile of cyanobacterium Nostoc sp. as a candidate for biodiesel production.

Methods: Collection was performed from soils and waters of Kharg Island in Persian Gulf. After purification, samples were incubated in BG110 culure medium at 25 ºC and constant light of 60 µE m-2s-1 and pH 7. Identification was carried out morphologically; and growth rate and pigments measured spectrophotometrically. The total amount of lipid, aminoacids and fatty acids profile and were measured by FTIR, HPLC and GC respectively.

Results: Allophycocyanin, Phycocyanin and Phycoerythrin of Nostoc sp. was 2.38, 5.37 and 5.05 mg mg dw-1, respectively. The amount of amino acid Nostoc sp. was about 0.73 mM gFw-1.The profile of fatty acids included Myrestic acid (1.58%), Palmitic acid(5.51%), stearic acid (2.3%), Palmitoleic acid(o%), Oleic acid (53.58%), Linoleic acid(11.18%), Linolenic acid(5.58%).

Conclusion: According to the results, Nostoc sp. can compete with other cyanobacteria for biodiesel production.

Keywords: Biodiesel, Cyanobacteria, Fatty acids, Growth, Phycobiliprotein.

P685: **Isolation of Rhizobium leguminosarum from clover and optimization of its culture for production of poly beta hydroxybutyrate**

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Background and Aim: Bacterial strains are able to produce biopolymers that are used as new resources for different industries. Many microorganisms are used in industries, but purpose are microorganisms that are suitable to produce cheaper biopolymers with more output. Among biopolymers that are used in industries, poly-beta-hydroxy butyrate (PHB) is more significant and practical, for example, in the production of biological biodegradable plastics and food industries. In this study we avaluated the ability of Rhizobium bacteria for production of PHB isolated from the roots of white and red clovers of Tonekabon city in Iran.

Methods: Sampling was conducted from 6 different regions. After culturing and purifying the bacteria, the differential biochemical tests were used to identify them and Rhizobium colonies isolated from each of 6 regions (R1, R2, R3, R4, R5, R6) were investigated. Medium YEM (Yeast Extract Monnitol) was used to isolate the bacteria, then the PHB production was investigated by using different carbon sources (glucose, maltose, galactose and starch). DCW and DCB were measured.

Results: Results showed that from the 6 isolated colonies, 4 colonies (R1, R2, R4, R6) showed the highest production of PHB that produced more than 10 mg/l PHB, and among these isolates only R1 is produced 32.4 mg/l PHB. After adding different carbon sources, production in the presence of starch was the highest and in the presence of glucose was the lowest. R1 in the presence of starch and maltose showed a high production, while the cell biomass (DCB) for this sample in the presence of glucose was the highest.

Conclusion: Based on similar studies in Iran and other countries, and based on studies of industrial strains producing PHB, bacteria isolated in this study, are one of the most important producers of PHB and can be used in many different industries.

Keywords: Rhizobium leguminosarum, clover, poly beta hydroxybutyrate.

P686: **Study of Morphological and Biochemical Alteration of Pasargadae Stone Monument Due to Deteriorating Microorganisms**

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Background and Aim: Stone monuments have the largest percentage of cultural heritage in the world. These stone monuments are exposed to many different types of climatic factors such as U.V. irradiation, humidity and temperature. Furthermore, stone monuments are also attacked by different types of macroorganisms and microorganisms like cyanobacteria, yeasts, some kind of algae, many types of molds. Microorganisms play an important role in biodeterioratin of stones. Pasargadae, established by Cyrus the Great in the 6th century BC, is the first dynastic capital of the Achaemenid Empire.

Methods: Stereomicroscope and scanning electron microscope was used to study morphological alteration of stone surfaces. XRD technique is carried out to map C, Ca, Si elements on the stones and assay biochemical alteration of stone surfaces.

Results: Different kind of physico-chemical deterioration was observed. The distribution of elements was affected by presence of biodeteriorating agents. After documentation of morphological alteration and type of them, isolation and characterization of deteriorating organisms should be done.

Conclusion: Biological studies before any restoration must be carried out to introduce the best technique for conservation.

Keywords: Biodeterioration, Pasargadae, Microorganisms, SEM, XRD

P687: **Molasses medium optimization for optimal bacterium Rhodococcus ruber strain KE1 growth using Taguchi experiment design method**

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Background and Aim: Oil contaminated soil causes many environmental concerns. Various procedures are being used for treatment of these pollutants among which biodegradation has a magnificent role in oil pollutions remediation. Many oil-degrading bacteria have been isolated and identified in different studies. Rhodococcus ruber strain KE1 which was used in this study, is a bacterial strain with extensive ability to degrade hydrocarbon compounds. For large scale applications, different industrial media such as beet molasses could be utilized for proliferating bacterial cells and knowledge about optimal conditions for culturing these bacteria in these industrial culture media is very important.

Methods: In this study, Nitrogen, Phosphorous and Carbon sources addition to the beet molasses medium was optimized by Taguchi experiment design method.The experiment was designed and done through Orthogonal array L8 which consists of three factors including Nitrogen [Four levels: Control, (NH4)2SO4, NaNo3 and NH4No3], Phosphorous (two levels: Control and Na2HPO4.2H2O) and Carbon (two levels: Control and Glycerol) sources.

Results: Analysis of results with Minitab software showed that in contrast to other related levels addition of (NH4)2SO4 and Na2HPO4.2H2O to the culture medium can significantly improve the growth of the strain KE1.

Conclusion: The bacterium also showed better growth in the presence of glycerol as a complementary carbon source; however the growth enhancement was not very substantial.

Keywords: Beet Molasses, Optimization, Rhodococcus ruber, Taguchi method

P688: **chemotactic behavior of Arcobacter butzleri toward nonpolar amino acids**

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Background and Aim: The genus Arcobacter , belong to the family of campylobacteraceae . Arcobacter spp. Are Gram negative , curved rod, motile by single polar unsheated flagellum. Arcobacter spp have been isolated from,wastewater , surfacewater or from food of animal origin especially from poultry , milk ,mussels and fecal samples of different animal species.Arcobacter butzleri is the most common species of genus that has been considered as the emerging and zoonotic foodborn pathogen.. As chemotaxis may be important for colonization or play a role in virulence factor for microorganisms and little is know about chemotactic behavior of Arcobacter butzleri we conducted this study.

Methods: We used capillary assays method that was first ever method developed and used for monitoring the bacterial chemotaxis.in this study we tested some none polar amino acids: Alanine,Leucine,tryptophan, Proline,Glycine,methionine.first we placed 10 ml of culture that contain 1.5 ×108 CFU/ml bacterial cells in the plate.then inserted 15 µl of 10 mM amino acid solution in Pasteur pipette in the culture and 15 µl PBS in another Pasteur pippet and suspend the third empty Pasteur pipette in the culture. then sucked out droplet from contents of each Pasteur pipettes during by 15 minutes intervals during 60 minutes. then plated each droplet on CAMP agar plates.we incubated plates at 25˚c for 48 hours and then counted the colonies.

Results: After 48 hours we observed increasing in the number of colonies during 0,15,30,45,60minutes for Alanine , leucine ,tryptophane and methionine.the most increased colonies respectively for the leucine and Alanine and tryptophane .methionine has been shown weak increased number of colonies,while we observed decline in the number of the colonies for proline and there have been no changes about numbers of colonies for glycine

Conclusion: all tested amino acids except glycine and proline showed positive chemotactic response . leucine is the strongest chemoattractant and respectively Alanine ,Tryptophan,Methionine. proline, acted as a repellent and Glycine showed no chemotactic response.

Keywords: Amino acids,chemotaxis.Arcobacter butzleri

P689: **Resistance pattern to antibiotics and heavy metals associated with polyphosphate accumulation in a strain of Pseudomonas aeruginosa**

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Background and Aim: The resistance pattern in microorganisms is different and the most of resistance genes has located on¬ Extrachromosomal site. Environmental microorganisms have high resistance to heavy metals. In some bacteria multi resistance to metals and antibiotics has been reported. This problem may be hazardous for salinity and public health because¬ these traits could be transferred between microbes in the environments. Phosphate accumulating bacteria tolerate toxic cations and positive charge complexes. In this research was focused relationship between phosphate accumulation and antibiotic and heavy metal resistance in order to introduce a type of detoxification and special resistance pattern.

Methods: The Sample was isolated from an area near coal tar refinery, located around Isfahan (Iran). Identification tests by biochemical method and 16SrDNA were done on it. Then, studies on the bacterium indicated ability to phosphate uptake and accumulation. At the following study, resistance to 200 ppm of NaVO3 ,Na2WO4 ,ZnCl2, MnCl2 ,CoCl2 ,FeCl2 ,CuSO4 ,Ag2SO4 ,CdCl2 ,Pb(NO3)2 ,Na2 MoO4 ,NiCl2 ,HgCl2 ,K2CrO4 was performed. All resistance studies were conducted on nutrient agar containing different phosphate concentrations. The samples were cultured by pour plate method and antibiotic resistance determined by disc diffusion on the basis of Kirby and Bauer method on the nutrient agar with the different phosphate amounts. The antibiotic disc using Streptomycin (10µg/disc), Rifampicin (30µg/disc), Ampicilin (10µg/disc), Carbencilin (100µg/disc), Chloramphenicol (100µg/disc), Novobiocin (30µg/disc), Polymyxin B (300µg/disc), Tetracycline (30µg/disc), Penicillin (30µg/disc), Erythromycin (15µg/disc).

Results: Identification tests showed similarity this strain to pseudomonas aeruginosa. The results indicated that among 14 experimented heavy metals, resistance to Mn2+, Co2+, Cd2+, Cro42- and Ni2+ increased with excessive phosphate concentration. MIC tests for antibiotics also showed high resistance to streptomycin at presence phosphate.

Conclusion: cationic and charged materials bands with polyanionic compounds as polyphosphate. Some of antibiotics and toxic metals were neutralized by polyphosphate accumulating bacteria. This strain isolated from soils contaminated able to phosphate uptake and accumulating and probably it has created resistance to metals and antibiotics.

Keywords: phosphate accumulation, Antibiotic resistance, toxic metal resistance

P690: **Polymerase chain reaction (PCR) detection of cyanobacteria in Anzali lagoon**

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Background and Aim: Anzali lagoon is one of the important aquatic environment in Iran. Cyanobacteria are ubiquitous organism that can be found in many diversified aquatic environments. There's two path to detection cyanobacteria in the aquatic reservoir, first is traditional methods such culture and morphological characteristics the second is molecular detection.

Methods: The specimens (20) of surface water were collected in 1.5 Lt bottles, Genomic cyanobacteria DNA was extrac ted by DNG-plus kit . In this study selected a universal primers (CYA359F – CYA781R) and amplified the gene target. PCR optimized and carry out sensitivity and specificity tests. Amplicon was cloned and sequenced by Dideoxy chain termination.

Results: The product of optimized PCR with 487 bp length correctly amplified and observed on electrophores gel. From 20 samples, 75% of them were positive for cyanobacteria.

Conclusion: The PCR technique used in this study due to the use of universal primer is more accurate and faster method for detecting of cyanobacteria.

Keywords: Cyanobacteria , PCR , Anzali lagoon

P691: **Determination of hu gene in Halobacillus litoralis**

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Background and Aim: Histone like protein HU is a small, basic heat stable protein which is Nucleoid-associated. The main function of HU protein is to inhibit DNA super coiling and to regulate DNA replication process. Halobacillus litoralis is moderately halophilic, gram-positive, heterotrophic bacterial strain. The Halobacillus litoralis genome encodes one histone-like protein that is similar to HBsu (HU protein of Bacillus subtilis). In this study we have identified hu gene with 320bp in Halobacillus litoralis.

Methods: This gene was amplified from Halobacillus litoralis with hbs forward primer (CAG TGA ATT CAT GAA CAA AAC AGA ACT TAC T) and hbs reverse primer (GAT GAA GCT TTT ATT TTC CGG CAA CTGC) by Polymerase Chain Reaction. The PCR product was electrophoresed on a 1.7% agarose gel and the band purified by DNA Extraction Kit and sequenced.

Results: Our result determined the sequence of hu gene in Halobacillus litoralis that is similar to hbsu in Bacillus subtilis. The HBsu PCR product has a size of nearly 300bp and H.litoralis PCR product size is approximately 320bp.

Conclusion: This result demonstrated the similarity between hu gene in Halobacillus litoralis and hbsu gene in Bacillus subtilis

Keywords: Halobacillus litoralis, hu gene, hbsu gene

P692: **Isolation and Identification of Heavy Metals Resistant Bacterium Producing Aantimicrobial Compound from Root of Avicennia marina, Evaluation of Antimicrobial Effect and measurement of heavy metal Resi**

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Background and Aim: In regard to increasing use of antibiotics the resistance of disease producing bacteria have been enhanced the researches about the usage of new antibiotics to control bacterial diseases. Beside in some environmental bacteria multi resistance to metals and antibiotic production has been reported. The aim of this study is isolation and identification the bacterium producing antimicrobial agent isolated from Root of Avicennia marina in Qeshm island, the evaluation of antimicrobial effects and heavy metal resistance of this bacterium.

Methods: In this study, Sampling was performed from soils around the Root of Avicennia marina in Qeshm Island and bacteria were isolated by serial dilution method on nutrient agar. The bacteria were grown in broth medium and the best bacterium producing antimicrobial agent was selected based on disk diffusion tests against standard microorganisms including gram negative bacteria, gram positive bacteria, yeast and also aspergillus niger. Then, identification was performed by morphological and biochemical tests and 16s rDNA analysis method. For investigation of bacterium resistance to heavy metal, resistance up to 400 ppm of ZnCl2, CoCl2, FeCl2, CuSO4, Ag2SO4, Pb(NO3)2 , NiCl2 , K2CrO4 and CaCl2 done.

Results: Results of identification showed that the isolated bacterium is similar to Brevibacillus agri. This bacterium is an obligate aerobic, gram positive, bacilli shape, catalase and oxidase-negative. The antimicrobial component is polar and showed a broad antimicrobial effect on the laboratory standard strains. Among 9 experimented heavy metals, this bacterium indicated maximum resistance to Cu2+, Co2+, and Ni2+ up to 400ppm and resistance to Pb2+, CrO42- and Fe2+ between 200-300ppm was obtained. But, growth at presence of Ag+ and Ca2+ was not observed.

Conclusion: Our study showed that the isolated bacterium has excellent antimicrobial activity against all tested standard microorganisms. High antimicrobial effects of the bacterium are evident therefore investigation on antagonistic effect of this compound on other bacteria and its identification are purposed. Although low concentrations of heavy metals is essential for growth of bacteria, but, high heavy metals level lead to inhibit growth. The presence of these bacteria, due to property has closely relationship with antibiotic resistance can be hazardous for community health.

Keywords: Brevibacillus agri, Avicennia marina, heavy metal resistance

P693: **Effects of heavy metal pollution on bacterial diversity in eastern area rhizosphere of Nayband mangrove forests**

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Background and Aim: Mangrove forests are among the most important aquatic ecosystem which provide not only a significant productive ecosystem but also are very important in biodegradation of organic material in tropical and semi-tropical sea shores. Nayband National Marine Park ecosystem as a biospherical reservoir is an important Mangrove forest in Persian Gulf. Because of high level of petrochemical activities in the Parse_e_Jonobi region there is a very high potential of contamination with petro-pollutant and heavy metals. Microorganisms play an essential role in biodegradation and mineralization activities and biological stability and functionality of these forests. Microbial biodiversity is a biomarker for determining the health condition of ecosystem. Ribosomal Intergenic Spacer analysis – RISA- as a rapid and low cost method is suitable for this purpose. This study was aimed to investigate the effects of heavy metal contamination on rhizospheric bacterial biodiversity of mangrove forests in Nayband region of Persian Gulf in Iran.

Methods: The samples were taken from superficial soil and sediments of mangrove roots from three spot in an area of 1m2 in the rhizosphere of eastern area of Nayband Gulf and mixed as one sample. Atomic adsorption was used to measure the heavy metal content. Direct DNA extraction was done from sediments and treatment by SDS and proteinase K, then introduced to RISA-PCR with 1406f, 5' GYACACACCGCCCGT 3' (universal, 16SrRNA gene), and 23Sr, 5'GGGTTBCCCCATTCRG 3' (bacterial-specific,23SrRNA gene) primers. The RISA-PCR product were subjected to electrophoresis with polyacrylamide gel (5%) and stained with gel red dye. The image of the gel was analyzed with Fire reader (UVITECH) and the Shannon-Weiner index (H') was obtained.

Results: The content of Cu, Zn and Mn in eastern area were 10.81, 49.54 and 34.05 ppm respectively. Fire reader analysis depicted that Shannon-Weiner index of eastern area was 0.577, while it was 0.637 for western area.

Conclusion: The value of Zn was rather higher than normal areas of other mangrove forests in the world which means that this area should classified as endanger and polluted area. The high measures of Cu, Zn and Mn for eastern area are due to this fact that this area is a semi-close area that does not have a good access to sea waters of Persian Gulf. The biodiversity index of this area is another biomarker of the endangering health of this ecosystem and another evidence of pollution in this area. H' index value signify the health value which the lower amount represent the lower health of an environment. H' Index of Florida mangrove forest was reported as 3.88 on Ln basis. But in the case of eastern part of Nayband forest it was quite lower. It is owing to this fact that the petrochemical and heavy metal pollution in this area affect the mangrove roots and consequently the quantity and quality of secreted substances from roots., so bacterial diversity that feeds from these substances will reduce. The results show that this area is in danger and need immediate action to control the contamination and recovering the ecosystem.

Keywords: RISA, Biodiversity, Mangrove, Heavy metal, Contamination

P694: **Screening and molecular identification of phosphate solubilizing bacteria from barley and wheat field in Fars Province**

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Background and Aim: Phosphate solubilizing bacteria include bacteria that provide phosphorus for growing plants which are observed in various forms in different groups of soils. These groups of microorganisms are capable to produce different inorganic acids using phosphorus. Hence the aim of this study was screening of the PSB from barley and wheat fields in order to identify and characterize the isolates.

Methods: In this study, 100 soil samples have been collected from wheat and barley farms. Then, based on serial dilution method, the phosphate solubilizing bacteria were isolated from soil samples on NRIP medium and the isolates with clear zone were characterized using phenotypic and molecular tests. In addition, the frequency of occurrence for isolated microorganisms evaluated using SPSS.

Results: In the present study the results from molecular identification indicated that totally 6 bacteria from wheat farms and 9 bacteria from barley farms isolated. The isolates from wheat were belong to: Alcaligenes faecalis strain IAM 12369, Klebsiella pneumonia subsp. Ozaenae strain ATCC 11296, Raoultella planticola strain ATCC 33531, Entrobacter kobei strain CIP 105566 and the others were belong to the barely field and they were characterized as: Entrobacter ludwigii Strain EN -119, Entrobacter kobei strain CIP 105566, Glaciecola agarilytica strain NO2 and Klebsiella variicola strain F2R9. The results obtained from statistical analysis indicated that, there is no significant difference between wheat and barley samples.

Conclusion: Although there are no differences between the fields it could be concluded that it is because of the identical place of sampling. In addition, the molecular identification indicated that most of the bacteria were belong to the family Enterobacteriaceae.

Keywords: Screening, Phosphate solubilizing bacteria, NRIP medium, molecular identification

P695: **Antimicrobial Resistance of Enterobacteriaceae Isolated of Karoun River in Iran**

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Background and Aim: Karoun River originates from the Zagres mountains and after travelling for 400 km enters into the Khouzestan plain. Preservation of the Karoun, the fresh water resource, is of high importance in Khuzestan province with its rapid growth of population and agricultural and industrial activities. The aim of this study was to determine antibiotic resistance of Enterobacteriaceae isolates form Karoun River in Iran.

Methods: Sample collection: A total of 300 water samples were obtained from Gotvand,Shushtar, Karoun river for two months (April to May 2011). Bacterial isolation: According to the standard dilution method, 0.01 from each concentration was plated onto MacConkey agar plates and incubated at 37°C for 24 h. All samples isolates were examined in the API 20E Kit. Among the isolates, 287 (65%) are Escherichia coli, 162 (27%) Enterobacter aeogenes,73 (12.16%) Citrobacter freundii, 58 (9.66%) Proteus vulgaris and 20 (3.3%) Salmonella typhi. For the antibiotic susceptibility testing disk diffusion method was used. Enterobacteriaceae genera were tested against twenty antibiotics: Ampicillin,Carbencillin,Methicillin,Piperacillin,Cephalothin,Cefotaxime,Ceftriaxone,Vancomycin, Amikacin,Tobramycin,Ofloxacin,Kanamycin,Tetracycline,Erythromycin,Clindamycin,Norfloxacin, Trimethoprim/Sulfamethoxazole,Nitrofurantoin and Chloramphenicol, Amoxycillin/Clavulanic acid.

Results: All Enterobacteriaceae isolates exhibited 100% resistance to Ampicillin, Carbenicillin, Methicillin, Vancomycin,Erythromycin,Clindamycin,Trimethoprim/Sulfamethoxazole and Tetracycline. Also, they failed to exhibit resistance to Norfloxacin and Ofloxacin.

Conclusion: Multiple antibiotic resistance (MAR) in enteric bacterial isolates from all the sites due to sewage discharge and input from other human sources along the karoun branch.

Keywords: Key words: Antibiotic resistance, Enterobacteriaceae, Karoun River Iran

P696: **Identification of Vibrio parahaemolyticus strain (4146104) in the coastal Busheher, Iran**

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Background and Aim: Vibrios are associated with live seafood as they form part of the indigenous microflora of the marine environment. Genetic differences in clinical and environmental strains of Vibrio parahaemolyticus have been widely used as criteria in identifying pathogenic isolates. However, few studies have been carried out to assess the differences in biochemical characteristics of V. parahaemolyticus isolates from human and environmental sources. V. parahaemolyticus strains of a few specific serotypes, probably derived from a common clonal ancestor, have lately caused a pandemic of gastroenteritis. The aim of this study was to the identification of V. parahaemolyticus in the coastal Busheher in the Persian Gulf,Iran.

Methods: The area of study is located in the coastal of Bushehr in Persian Gulf, which had been chosen as sampling sites. A total of one hundred and eigt samples water sea were collected and analyzed, during April to September 2012. Then, on the basis of colony shape on Thiosulphate Citrate Bile Salt sucrose (TCBS) agar,catalase activities, motility and sensitivity to vibriostatic and growth in peptone water at different NaCl concentrations (1% - 2%). A more complete genus identification was obtained using the API 20E test.

Results: Water sea samples (108), V. parahaemolyticus strain 4146104 (21) was found in the water of the coastal zones. Study showed that most was suggested that V. parahaemolyticus is commonly found in the water sea , mussels and mollusca.

Conclusion: The isolation of some potential pathogenic V. parahaemolyticus species shows the importance of V. parahaemolyticus research identification to estimate water quality and to avoid transmission of infection to man and to other marine organism.

Keywords: Pathogenic, Vibrio parahaemolyticus, Food Poisoning , Sea water,Persian Gulf

P697: **Isolation of Rhizobium leguminosarum from clover and optimization of its culture for production of poly beta hydroxybutyrate.**

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Background and Aim: Bacterial strains are able to produce biopolymers that are used as new resources for different industries. Many microorganisms are used in industries, but purpose are microorganisms that are suitable to produce cheaper biopolymers with more output. Among biopolymers that are used in industries, poly-beta-hydroxy butyrate (PHB) is more significant and practical, for example, in the production of Biological biodegradable plastics and food industries. In this study we avaluated the ability of Rhizobium bacteria for production of PHB isolated from the roots of white and red clovers of Tonekabon city in Iran.

Methods: Sampling was conducted from 6 different regions. After culturing and purifying the bacteria, the differential biochemical tests were used to identify them and Rhizobium colonies isolated from each of 6regions (R1, R2, R3, R4, R5, R6) were investigated. Medium YEM (Yeast Extract Monnitol) was used to isolate the bacteria, then the PHB production was investigated by using different carbon sources (glucose, maltose, galactose and starch). DCW and DCB were measured.

Results: Results showed that from the 6 isolated colonies, 4 colonies (R1, R4, R6, R2) showed the highest production of PHB that produced more than 10 mg/l PHB, and among these isolates only R1 is produced 32.4 mg/l PHB. After adding different carbon sources, production in the presence of starch was the highest and in the presence of glucose was the lowest. R1 in the presence of starch and maltose showed a high production, while the cell biomass (DCB) for this sample in the presence of glucose was the highest.

Conclusion: Based on similar studies in Iran and other countries, and based on studies of industrial strains producing PHB, bacteria isolated in this study, are one of the most important producers of PHB and can be used in many different industries.

Keywords: Rhizobium leguminosarum, clover, poly beta hydroxybutyrate.

P698: **Effect of Nacl and PH factors on dipicolinic acid (DPA) formation by Bacillus subtilis PTCC1023**

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Background and Aim: Dipicolinic acid (pyridne 2, 6 dicarboxylic acid) is a major component of bacterial spore and is unique in that it has been found only in bacterial endospore comprising 5-15% of the spore dry weight

Methods: In this study of colorimetric Assay the color complex fomed by interaction of ferrous iron with dipicolinic acid was used. In this study the effects of environmental stresses(PH , Nacl) on the production of DPA by Bacillus subtilis PTCC1023strains were evaluated.

Results: Results showed that the amount of DPA in B.subtilis decreased upon increasing acidic condition and salt in the medium . DPA formation was more significant at alkaline pH rather than acidic pH.

Conclusion: In stressful condition environments DPA formation reduced significantly and this properties can beuse in food and dairy industries to prevent spreading at Bacillus stains.

Keywords: colorimetric, spore, Dipicolinic acid, Bacillus subtilis PTCC1023

P699: **Optimization of Cr(VI) bioreduction using Response Surface Methodology(RSM) in In vitro condition**

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Background and Aim: Chromiume is an intermediate element with two oxidation states(III & VI), the hexavalent form is more harmful in environment due to its solubility in water and In this study we work on removal of Cr(VI),. we found that 3 important parameters such as pH , agitate rate and glucose concentration have the most influence in removal of Cr(VI) so we try to optimize this parameters and survey the effect of them together in, In vitro condition. In this paper, response surface methodology (RSM) involve central composite design(CCD) have been applied to design the experiments to evaluate the interactive effects of three effective variables: pH (1.0–8.0), agitation (0–250 rpm) and glucose concentration (0-3%) on biosorption/bioreduction of Cr (VI) ions with permeabilized native bacterial cells . 20 experiments were designed towards the construction of a quadratic model. Very high regression coefficient between the variables and the responses (R2 =0.91) indicates evaluation of experimental data by second order polynomial regression model.

Methods: A Cr (VI) resistant bacteria strain previously isolated from chromium mine and identification of this strain was done by partial sequencing of 16S rDNA gene. bacteria strain was cultured at 180rpm and 37 ◦C in pH =7,medium consist of 1% tryptone; 0.5% yeast extract; and 1% NaCl dissolved in distillated water.

Results: Response surface methodology is a combination of mathematical and statistical techniques used for developing, improving and optimizing the processes. In the present study we have permeabilized 2mL bacteria biomass(OD660=2.57) with EDTA. finally we found that this amount of biomass have high ability to remove 100mg/l concentration of Cr(VI) in 2hr. result shows that when EDTA was used in permeabilizing process, chromium removal improve so we derive that mechanism of removal confirms enzymatic reduction. cyclic voltametery diagrams shows our hypothesis is true and chromate reductase enzyme plays an important role in reduction of Cr(VI).

Conclusion: The present study shows that response surface methodology is a fast and error free approach for optimization of media composition to obtain the best performance of CKCr-8 for Cr (VI) removal.results shows that pH and agitation rate played a determinant role in uptaking Cr(VI). On the basis of this method using the CCD design, found that combination of pH, agitation rate and concentration of glucose in constant biomass dosage have significant effect on bioreduction of Cr (VI) in In vitro condition.

Keywords: bioremediation,response surface methodology,reductase

P700: **Molecular identification and detection of Campylobacter Jejuni in surface waters of Rasht, Iran**

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Background and Aim: Campylobacter as an infectious agent with zoonotic origin is known as an important cause of gastroenteritis in humans. Humans are infected through oral-fecal route and contaminated waters. In order to sanitary control of the disease in water resources, determination of this bacterium is valuable. The aim of this study is molecular epidemiology of Campylobacter jejuni in surface waters of Rasht, Iran.

Methods: This cross-sectional study was done on 45 samples surface waters of Rasht. Purification of DNA from water samples was performed based on STET method. Amplification of Campylobacter Jejuni gene was done by specific primer. Then PCR product with 735 bp length was observed through agarose gel electrophoresis.

Results: In terms of Campylobacter jejuni contamination, seven of the total samples (15.55%) were positive. Comparison of the amplified sequence of Campylobacter jejuni with the standard sequence in the NCBI resources showed highest homology

Conclusion: PCR technique is as a precise and rapid screening method for the detection of Campylobacter jejuni on the surface waters. Prevalence of Campylobacter jejuni in surface water of Rasht is considerable. Because this method does not need any microbe culture and expensive antibody, therefore, it is recommended to determine this bacterium in surface water samples.

Keywords: Campylobacter jejuni, surface waters, PCR, Rasht

P701: **Evaluation of airborne fungal contamination of ENT ward, after the sterilization and during the operation at Imam Reza hospital in Mashhad-2012**

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Background and Aim: The evaluation of the amount, types, and diversity of bio contamination in hospital rooms especially operation room is very important to control and prevent hospital acquired infections (HAI). Because by studying of dissemination, spread, and movement of airborne fungal spores, particularly the pathogenic ones in the atmosphere by controlling them, can decrease the acquired hospital infections. The objective of this study was the assessment of rate fungal contamination air indoor in the ENT ward of Imam Reza hospital in Mashhad -2012.

Methods: In this cross-sectional study, during the autumn, 30 samples of air of the ENT ward, after the sterilization and during operation, were collected by open plate technique (on Sabouraud dextrose agar) Samples immediately transported to the laboratory. Then plates were placed in incubator for 7-10 days at 32.5°C.The cultures were examined by microscopic and macroscopic methods.

Results: Our results showed, 63.34% of the samples were positive for fungal contamination. The most fungi were observed in the samples after sterilization (disinfection with ozone) by Aspergillus fumigatus and Penicilliumspp and in the samples during operation, yeast and Aspergillus flavus.

Conclusion: According to the results, the sterilization (disinfection with ozone)ENT ward should modify. For controlling of fungal contamination in ENT ward and various wards of the hospitals especially those have immunocompromised patients or other patients with predisposing factors, suitable filtration and ventilation air and proper disinfection of the wards seem to be necessary.

Keywords: Hospital wards, ENT ward, airborne fungi, Mashhad

P702: **Isolation and Rapid identification of Clostridium perfringenes by using Fluorogenic substrates and membrane filter from Cheshme kile river (Mazandaran Province)**

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Background and Aim: The sulfite-reducing clostridium group, including Clostridium perfringens, has been shown to be of value in assessing fecal pollution of surface and ground water and estuarine and ocean environments. C. perfringens is an anaerobic, gram-positive, spore-forming bacillus that is capable of surviving in soil and water for extended periods of time. Clostridial spores survive longer than coliforms, Escherichia coli or enterococci and are consequently used as an indicator of past faecal pollution. The spores are not always inactivated by chlorination but are not a hazard to health in potable water. Although the resistance of spores of C. perfringens gives them similarities to Cryptosporidium and Giardia they are not reliable indicators of the presence of these parasites in drinking or recreational waters. In environmental samples such as surface waters a wide range of Clostridium species may be present. The aim of this study was to investigate fecal contamination of surface waters with Clostridium perfringens in this region.

Methods: Water samples were collected for processing from river and surface water storage locations. Selection for sulfite-reducing clostridia including C. perfringens spores was undertaken by heat treatment of water samples at 70°C for 20 min to kill vegetative cells. Heat treated samples were then filtered through a 0.45-mm-poresize cellulose-acetate membrane filter. Membranes were then transferred onto freshly prepared TSC or TSC-MUP agar plates and incubated at 37°C for 24 h in an anaerobic workstation with a gas mixture of 10% hydrogen in 90% nitrogen. The samples were examined under UV light.

Results: In this study, 10 water samples taken from locations known to be impacted by fecal contamination were assessed. Samples were heat treated and allowed to cool, and following filtration, membranes were placed onto TSC-MUP agar. Plates were incubated anaerobically at 35°C for 24 h. A total of 10 presumptive C. perfringens isolates were transferred to TSC-MUP agar and 6 of 10 isolates fluoresced on TSC-MUP agar.

Conclusion: Fluorescence on TSC-MUP agar was difficult to interpret under UV due to diffusion of 4-methyl-umbelliferone, especially when colonies were clustered together or when they were present in large numbers. The data confirmed that although TSC-MUP agar is sensitive, it also has a high false-positive rate.

Keywords: Isolation, Clostridium perfringenes, Fluorogenic substrates, Mazandaran Province

P703: **Bioavailability of Effective Microbial Population of Tabriz Regions Soils on the Degradation of Polycyclic Aromatic Hydrocarbons**

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Background and Aim: Aromatic polycyclic s hydrocarbons such as other anthracene, naphthalene and phenatherene are main poisons pollutants and carcinogenic which are dis changed into environment in the waste water of oil, petrochemical, dye, rubber, pharmaceutical and plastic industries. These pollutants would affect the environment and cause serious damage to soil, water and living bodies. In order to eliminate these contaminants some biological methods and also using microorganisms present in soil due to its low cost and relatively little danger have been utilized and preferred to other methods. The existing microorganisms in soil consume hydrocarbons as source of carbon and energy and consequently produce water, CO2, biomass and harmless substances.

Methods: In the present study the samples were prepared from contaminated soils of Tabriz refinery and different places in Tabriz and the culture was carried out in YGM agar and starch casein agar and 100 pure microbe colony were isolated. 1000 mgl-1 of naphthalene, anthracens and phenanthrene were prepared in muller hinton broth and constant amounts of above bacteria were added separately and kept under incubation at 28 and 130r.p.m for one week. The rate of destruction of above hydrocarbons was evaluated by spectrophotometer and UV-Chromatography.

Results: The results should that 94 bacteria capable dissociating naphthalene were isolated and the rate of destruction was 3.5-92.9% and 87 bacteria capable dissociating phenanthrene and the rate of destruction was 3.2-93.8% and 90 bacteria capable dissociating anthracene and the rate of destruction was 3.4-82.6% and the 19 bacteria for naphthalene, 12 bacteria for phenanthrene and 13 bacteria for anthracene were degraded more than 50%.

Conclusion: Bioremediation is a natural process by which pollutants are recycled rather burying them. Bacteria have the most importance compared with other microorganisms because of their different reductive enzymes .Considering the results of the present study and conducted studies, soil bacteria, more or less, have the potential of reduction and destruction of polycyclic aromatic hydrocarbons.

Keywords: Soil microorganisms, Biodegradation, Soil pollution, Aromatic poisons, PCR

P704: **Isolation and Identification of Rhizosphere Soil Chitinolytic Bacteria and their Potential in Antifungal Biocontrol.**

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Background and Aim: Chitinases are a group of antifungal proteins that catalyze the hydrolytic cleavage of the β-1,4-glycoside bond present in the biopolymers of N-acetyl-D-glucosamine, mainly in chitin.Second only to cellulose, chitin is the most abundant polysaccharide world-wide. Many chitinases have been identified and characterized from various sources mainly from bacteria(for example: Enterobacter,Bacillus,Pseudomonas fluorescens)that can act as biological control of different phytopathogens. The aim of this study as to isolate chitinolytic bacteria from Rhizosphere soils and to screen their antagonistic activity against some fungal pathogens.

Methods: Samples were collected from Rhizosphere soil of north iran.Chitin degraders were isolated by serial dilutions of soil samples and plated on 0.5% colloidal chitin agar (CCA)medium.next the isolates capable of degrading chitin with distinct zone of clearance on CCA were selected. Chitinase assay was read at 585nm in a spectrophotometer. The antifungal activity was assayed in vitro by inhibiting the growth of phytopathogenic fungus (Fusarium solani)on potato dextrose agar (PDA) media. isolate was identified on morphological observation and 16SrRNA analysis.

Results: Only one isolate designated as Serratia liquefaciens new strain.was the most potent chitinolytic bacterial isolates.The srain exhibited a maximum chitinase production of 4.37 units/ml in colloidal chitin broth after 4 days of cultivation at 30°C.The results demonstrated that Serratia liquefaciens new stain isolate was the most effective isolates against tested phytopathogen,where the largest inhibition zone was 2.5 centimeter.

Conclusion: In recent years, much attention has been given to the antagonistic bacteria that can be used for biological control of pathogenic fungi. Several studies support the important role of chitinolytic bacteria as promising biological control agents for various phytopathogens.the chitinase enzyme from endemic bacteria can be directly applied for suppressing growth of living fungal hyphae.

Keywords: Antifungal activity, chitinase, F.solani, CCA ,PDA

P705: **Evaluation and Effect of carbon sources on Poly-beta-hydroxybutyrate PHB synthesis by Azotobacter vinelandii Isolated from Soils of Guilan Province North of Iran**

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Background and Aim: The purpose of this study was optimization of environment food ingredient and culture conditions to improve PHB produced by bacteria Azotobacter spp.

Methods: At first, using One-Factor-at- a- time,effect of different carbon sources, as well as incubation time of production was evaluated. Then, optimization in the best carbon sources was performed.

Results: Results showed that among carbon,Sucrose was the best source that could produce PHB. The best time for PHB production by sucrose was obtained in 48 hours. In general, between concentrations of (1 - 8% w/w) of carbon sources, the concentration of (2% w/w) resulted in highest production.

Conclusion: The highest PHB yield using carbon source among different kind of carbon sources occurred in the incubation time 48 hours. Almost similar trend Poly-beta-hydroxybutyrate accumulation was observed when maltose, mannitol, glucose were used as carbon sources in the media.

Keywords: Azotobacter vinelandii, Optimization, Poly-beta-hydroxybutyrate.

P706: **Biodegradation of Glyphosate herbicide by isolated halophilic bacteria from environmental samples from Qom Hoze-soltan lake**

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Background and Aim: Glyphosate (N-(phosphonomethyl)Glycine) is an organophosphorous pesticide with a high degree of dangerous effect towards the environment. We have investigated the biodegradation of halophilic bacteria isolated from Qom Hoze-Soltan lake.

Methods: The five of area around the Hoze Solthan lake was sampled. After sampling and bacterial isolation, native halophile straines grown in the presence of Glyphosate at a wavelength of 660 nm and also The disappearance of the Glyphosate in the control(Glyphosate herbicide) at a wavelength of 220 nm was determined. and selected preponderant isolate. Biochemical and antibiotic tests and The minimum inhibitory concentration test(MIC), for preponderant isolate was done. Analysis of Glyphosate Herbicide was performed by HPLC analysis after derivization with FMOC-Cl(9-Fluorenylmethyl-chlorformiat). Molecular identification was performed by 16S rRNA . Different physical and chemical parameters were optimized at different levels of the Taguchi method for preponderant isolate.

Results: From 10 halophilic isolates obtained, three isolates had the ability to grow in the presence of the Glyphosate Herbicide. the ability to isolate SH5 growth and reduce the amount of Herbicide that As degrading the herbicide chosen. The minimum inhibitory concentration equal to 1080 ppm. HPLC peak of Glyphosate were obtained at 16.567 (min) and HPLC peak of metabolite of degradation at 6.247 min .

Conclusion: According to the results of biochemical tests and antibiotic and molecular 16S rRNA, native halophilic isolates obtained were very similar to Salinicoccus sp.

Keywords:: glyphosate, Biodegradation, halophilic microorganisms

P707: **The effects of nutritional and environmental factors on growth pattern of Mycobacterium marinum CCUG 20988**

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Background and Aim: Mycobacterium marinum is a ubiquitous, slow growing nontuberculosis that has an optimal growth temperature of 30–32ºC. This organism can cause disseminated granulomatous infections in fish and other poikilothermic animals called 'fish TB'. Changes of growth pattern is a strategy by which microorganisms survive in the face of a changing or hostile environment, The aim of study was evaluation of changes in nutritional and environmental factors on growth pattern of M. marinum CCUG 20988

Methods: M. marinum CCUG 20988 was grown in 7H9 broth medium containing different concentrations of Tween 80 (50, 100, 150, 200, 250 and 300 µg/ml), Oleic Albumin Dextrose Catalase supplement (OADC), (2, 4, 6, and 8/ml) and glycerol (30, 35, 40, 45, and 50%). Also different pH (4, 5, 6, 7, 8 and 9), temperature conditions (30, 37, 40, 45 and 50ºC). The growth patterns of mycobacterium were measured at OD=600 nm and compared to standard growth curve.

Results: The obtained results showed that M. marinum CCUG 20988 growth was maximum at 200 µg/ml of Tween 80. Oleic Albumin Dextrose Catalase supplement at different concentrations did not showed significant effect in increasing growth pattern of M. marinum CCUG 20988. Decreasing glycerol concentration significantly decreased growth rate of M. marinum CCUG 20988. The best growth temperature and pH were 30 ?C and 7, respectively.

Conclusion: According to the results we can conclude that Tween 80 and glycerol in formulation of Middlebrook 7H9 Broth are very critical for M. marinum CCUG 20988 growth. But in contrast Oleic Albumin Dextrose Catalase supplement can be omitted from medium formulation due to not significant effect on growth pattern. Also, the optimum environmental conditions including temperature and pH were similar to the previous studies.

Keywords: Mycobacterium marinum CCUG 20988, growth pattern, environmental conditions

P708: **Phylogenic identification of laccase producing a strain fungus from bagass**

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Background and Aim: Laccases are an interesting group of multi copper enzymes, which have received much attention of researchers in last decades due to their ability to oxidize both phenolic and non-phenolic lignin related compounds as well as highly recalcitrant environmental pollutants. In this research our aim was isolation and identification of laccase producing microorganism from bagass As a source of lignocellulose.

Methods: In this research Laccase producing fungi was screened from soil around sugarcane root and bagass. Isolation and purification of fungi from collected samples was done on malt extract agar medium (MEA). In order to screen Laccase producing strains MEA containing 0.3 g l-1 ABTS [2, 2-azinobis (3-ethylbenzathiazoline-6-sulfonic acid)] was used. We used Trametes versicolor and Phanerochaete chrysosporium as positive and negative control of laccase assay, respectively. For phylogenic identification, one species was identified using PCR amplification and sequencing of the internal transcribed spacer 'ITS'regions of the ribosomal DNA and by basic morphology.

Results: According to morphological and molecular characteristic, isolated fungi had similarity to Alternaria . Therefore phylogenetic relationship of sequence of isolated fungi with related strains sequence was considered in gene bank using Blast. The comparative analysis of 5.8S rDNA sequence revealed that it was close to the members of Alternaria sp.

Conclusion: One efficient laccase producing fungus isolated from bagass

Keywords: laccase, Phylogenic identification, Alternaria

P709: **Study on antibacterial activity of leaf methanolic extract of Mespilus germanica against positive and negative bacterials**

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Background and Aim: Nowadays , due to the resistance of pathogenic microorganisms to antibiotics, more attention is to use of bioactive compounds of plant species. In most of the world, medicinal plants are used for antibacterial activity, antifungal and antiviral effects. The aim of this study was to evaluate the antibacterial activity of leaf methanolic extract of Mespilus germanica against positive and negative bacterials

Methods: In this research leaves of Mespilus germanica L. was collected in three altitudes (sea level, 1000 and 2000 meter) and methanolic extracts prepared by suxelet. Then the effect of metanolic extracts on four bacteria Escherichia coli(PTCC: 1399), methicillin-resistant Staphylococcus aureus(ATCC: 25925), Bacillus subtilis (PTCC: 1023), pseudomonas aeroginosa(PTCC: 1430) with MIC method was assayed.

Results: results showed that extracts have inhibitory effects on all four bacteria. Most effect was for Samples that were collected from heights of 1000 meter from sea level, on methicillin-resistant Staphylococcus aureus (MIC: 15.6 µg/ml).

Conclusion: this finds indicated that methanolic extracts of Mespilus germanica have antibacterial activity. For this reason we suggest more researches on the yields of identification and use of antibacterial components for treatment of diseases instead of antibiotics. Of course clinical application of this plant needs to more study and check on in vivo

Keywords: antibacterial activity, medicinal plants, methicillin-resistant Staphylococcus aureus, Bacillus subtilis, Escherichia coli, pseudomonas aeroginosa

P710: **Role Synergy Prickly Pear cacti The Stimulation Antimicrobial Activity Enterococcus durans Isolated Of Honey Stomach The Worker Honeybee**

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Background and Aim: Natural products perform various functions and many of them have interesting and useful biological activities and some the medicinal plants have become part of complementary medicine worldwide, because of their potential health benefits. This study concentrates on the role synergy prickly pear cacti the stimulation antibacterial activity Enterococcus durans isolated of honey stomach

Methods: samples of the honey stomach of the worker honeybee using selective medium MRS, under anaerobic condition using anaerobic jar with Anaerocult c gas packs at 37˚c was incubated and by biochemical tests and molecular analysis was isolated Enterococcus durans. After the overnight the broth was centrifuged 10000 rpm at 4˚c for 20 min. supernatant was used as a crude bacteriocin and they were evaluated using well diffusion method for antimicrobial activity against gram positive bacterial such as bacillus cereus, MRSA. Then the samples containing antimicrobial compound and prickly pear fruit (Ethanolic, Methanolic and water extracts) were added to wells at 25µl, 50µl, 75µl and 100µl concentration. The plates were then incubated at 37˚c for 24 hours and the activity was measured by the presence of inhibitory zone

Results: results showed that there are significant differences between all factors that were examined ($p<0.05$) and most inhibitory effect of ethanolic extracts of prickly pear cacti was on methicillin-resistant Staphylococcus aureus and Bacillus cereus (corona diameter mean: 25.83 ± 3.71 mm).

Conclusion: in this study, the synergy prickly pear fruit extract was found that stimulate antimicrobial activity Enterococcus durans isolated of honey stomach. Hence use of this fruit extracts can be effective at increasing the therapeutic effect and as well as increasing production of antibiotics produced by this genus of the bacteria

Keywords: prickly pear cacti, Enterococcus durans, Honey stomach, Synergy, Antimicrobial activity

P711: **Fungitoxic activity of the essential oil of Eryngo (Eryngium bungei Boiss.)**

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Background and Aim: The aims of this study were to examine the chemical composition of the essential oil isolated from seeds of Eryngo (Eryngium bungei Boiss.), and to test the efficacy of essential oil as an antifungal potential against Sclerotinia sclerotiorum (Lib.) de Bary. The oil revealed remarkable antifungal effect against S. sclerotiorum. Introduction Eryngium bungei Boiss. is a perennial plant of Apiaceae distributed in east of Iran. Sclerotinia sclerotiorum (Lib.) de Bary is a necrotrophic fungal pathogen causing disease in a wide range of plants. S. sclerotiorum is capable of colonizing over 400 plant species found worldwide. The majority of these species are dicotyledonous, although a number of agriculturally significant monocotyledonous plants are also hosts.

Methods: Plant materials: The seeds of E. bungei were collected in August 2012 from Oojan, suburb of Birjand, east of Iran. Isolation of the essential oil: The powdered seeds (100 g) of E. bungei were subjected to hydrodistillation using a Clevenger-type apparatus for 5 h to yield 0.25% of yellowish oil. GC–MS analysis: The essential oil was analyzed by GC/MS using a Hewlett Packard GC–MS (GC-7890A & MS-5975C) equipped with a HP-5 silica capillary column (30m×0.25mm, film thickness 0.25 µm) which was programmed as follows: 60°C (3 min), then 60°-230°C at 5°C/min and finally held isothermal at 230°C for 10 min. The carrier gas was helium (99.999%) at a flow rate of 1 mL/min. Diluted sample (1/20, v/v, in n-hexane) of 1.0 µl were injected manually in the splitless mode. The relative percentage of the essential oil constituents was expressed as percentages by peak area normalization. Fungitoxic assay: Petri dishes (90 mm diameter) containing 15 ml of PDA were used at 5 concentrations of essential oil (0.001, 0.01, 0.1, 1 and 10 mg/ml) in combination with the medium. The positive and negative controls were Amphotericin B (0.01 mg/ml), and distillated water in place of oil, respectively. The plates were inoculated with 6 mm plugs of 7-day-old cultures and were incubated at 25°C for 7 days, until the growth in the control plates reaches the edges of the plates. Growth inhibition of fungal strain was calculated as the percentage of inhibition of radial growth relative to the control, using the formula (Razavi and Zarrini, 2010): Mycelia growth inhibition (%) = [(Dc– Dt)/Dc] ×100% Where Dc and Dt are average diameters of fungal growth in the control and treatment groups, respectively.

Results: The GC-MS analysis determined 73 compounds, which represented 92.04% of total oil, were present in the oil containing mainly Chrysanthenyl Acetate (19.99%), Spathulenol (17.21%), Endo-Isofenchol (10.79%) and Alpha Pinene (5.05%). The essential oil (0.001, 0.01, 0.1, 1 and 10 mg/ml) showed potent inhibitory effect on the growth of S. sclerotiorum 76%, 88%, 99%, 100% and 100%, respectively; while Amphotericin B (0.01 mg/ml) showed 72% relative to the negative control.

Conclusion: The results demonstrate that the essential oil of Eryngium bungei Boiss. could become a potential alternative to synthetic fungicides for controlling certain important agricultural plant pathogenic fungi.

Keywords: essential oil, antifungal, Sclerotinia sclerotiorum, Endo-Isofenchol, Chrysanthenyl Acetate, Spathulenol

P712: **Antifungal effects of Eryngium bungei Boiss. root extracts on Sclerotinia scelerotiorum**

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Background and Aim: To evaluate the antifungal effects of Eryngium bungei Boiss. root extracts on Sclerotinia sclerotiorum (Lib.) de Bary, an experiment was performed at University of Mohaghegh Ardabili in 2012. Soxhlet extraction was done with n-hexane, dichloromethane and methanol solvents, respectively. All extracts have significant antifungal effects on S. sclerotiorum. Introduction Eryngium bungei Boiss. is a perennial plant of Apiaceae distributed in east of Iran with basal leaves undivided, spiny, pinnately to reticulately veined; involucral leaves at least twice as long as capitula, linear to lanceolate; bracts about as long as flowers; fruits, mostly entire. Sclerotinia sclerotiorum (Lib.) de Bary is one of the most devastating and cosmopolitan of plant pathogens. More than 60 names have been used to refer to diseases caused by this fungal pathogen including cottony rot, watery soft rot, stem rot, drop, crown rot, blossom blight and, perhaps most common, white mould. The fungus infects over 400 species of plants worldwide including important crops and numerous weeds.

Methods: Plant materials: The roots of E. bungei Boiss. were collected in August 2012 from Oojan, suburb of Birjand, east of Iran. The powdered dried roots of the plant were extracted using a soxhlet apparatus with n-hexane, dichloromethane and methanol solvents, respectively. The extracts were dried with a rotavapor apparatus. Antifungal assay: Petri dishes (90 mm diameter) containing 15 ml of PDA were used at 4 concentrations of each extract (0.001, 0.01, 0.1 and 1 mg/ml) in combination with the medium. The positive and negative controls were Amphotericin B (0.01 mg/ml), and distillated water in place of extracts, respectively. The plates were inoculated with 6 mm plugs of 7-day-old cultures and were incubated at 25°C for 7 days, until the growth in the control plates reaches the edges of the plates. Growth inhibition of fungal strain was calculated as the percentage of inhibition of radial growth relative to the control, using the formula (Razavi and Zarrini, 2010): Mycelia growth inhibition (%) = [(Dc–Dt)/Dc] ×100% Where Dc and Dt are average diameters of fungal growth in the control and treatment groups, respectively.

Results: The n-hexane, dichloromethane and methanol extracts (0.001, 0.01, 0.1, and 1 mg/ml) showed potent inhibitory effects on the growth of S. sclerotiorum (74%, 85%, 95%, and 99%), (70%, 84%, 93%, and 96%) and (64%, 74%, 83%, and 88%), respectively; while Amphotericin B (0.01 mg/ml) showed 72% relative to the negative control.

Conclusion: E. bungei Boiss. root extracts have significant antifungal effects on S. sclerotiorum. Between the extracts, n-hexane and dichloromethane had more intensive effects, respectively. Since the plant, like other genus of Apiaceae having secondary metabolites such as phenolic compounds, coumarins, alkaloids and terpenoids; its antifungal effects can be attributed to the presence of these compounds in the plant. So its property is recommended in controlling certain important agricultural plant pathogenic fungi.

Keywords: Eryngium L., antifungal, Sclerotinia sclerotiorum, methanol extract

P713: **Detection of campylobacter jejuni**

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Background and Aim: campylobacter is a gram negative, spiral and microaerophilic bacteria that it is a common diarrhea in humans cause of acute disease. current diagnostic method for detecting campylobacter jejuni infection include culture, PCR, serum antibody detection. Hippurate hydrolysis is the most commonly method to diagnose campylobacter infection because of its simple, rapid, specific and accurate characters. the aim of this study is evaluation of Hippurate hydrolysis test compared with culture for diagnosis of campylobacter jejuni.

Methods: campylobacter used in this study was isolated fecal samples from different patients with acute diarrhea disease(n= . Stool diluted in 500 µl PBS and 100 µl added to thioglycollate medium was incubated over night under microaerophilic environment at 42°C.then cultured in charcoal and campylobacter blood agar base medium and 10 µl was tested for Hippurate hydrolysis.

Results: 20 fecal samples were selected from different patients with acute diarrhea. then identical C,jejuni were recovered growth was pure on 8 charcoal medium.9 fecal sample were positive for Hippurate hydrolysis

Conclusion: various test for diagnosis of C.jejuni are discussed. The best methods for detection C.jejuni infection is Hippurate hydrolysis

Keywords: campylobacter jejuni,Hippurate hydrolysis,culture

P714: **Enterobacter ludwigii a possible candidate for the bioremediation of multi-polluted environments by heavy metals**

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Background and Aim: Heavy metals are very toxic elements, which comprise the essential part of anthropogenic pollutants. Microorganisms have a high surface area-to volume ratio which provide a large contact area that can interact with metals in the surrounding environment. Moreover, remediation of toxic metals by bacteria offers a relatively inexpensive and efficient way for the decontamination of polluted environments. Therefore, the main goal of this study is evaluation of resistance threshold of selected bacteria to mercury and copper oxyanions.

Methods: In this study, isolated Enterobacter ludwigii, which have high resistance level to selenate and deposited in GeneBank database as accession JQ965666 was used. In order to resistance level determination of this isolate, MIC (Minimum Inhibitory Concentration) and MBC (Minimum Bactericidal Concentration) tests were performed. Assessment of growth capability at the presence of mercury and copper performed in the seperated modified-Luria Bertani broth medium which containing of 25-800 ppm HgCl2 l of bacterialμand 2-64 mM Cu2SO4. These tubes inoculated by 100 C,°suspension (set to McFarland Standard No.0.5). After incubation at 30 48-72h and 150rpm, each test tube which didn't have any observing growth was applied to MBC determination.

Results: MIC assays showed that this isolate have MIC equal to 50ppm and 8mM toward mercury and copper oxyanions, respectively. The results of MBC test showed that this bacteria has MBC equal to 100ppm for mercury and 8mM for copper.

Conclusion: The major effects of mercury poisoning are neurological and renal disturbances, as well as impairment of pulmonary function. Moreover, copper is an essential nutrient. However, excessive concentrations of this metal are well known to be toxic to most living organisms. Therefore,with this regard that our isolate has high tolerate to selenate (MIC 600mM) in contrast to many studies, and the data of present study showed relatively high resistance level to mercury and copper, it can be a possible candidate for bioremediation of polluted environments.

Keywords: microorganisms, Heavy metals, pollutants, resistance threshold

P715: **Isolation, Identification and characterization of Decolorizing bacteria from environmental samples**

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Background and Aim: Nowadays, drug, pulping, texture and other industries are developed in most of the countries. Currently the most important dangerous sewage is pigmented sewage, because they have complex structures such as azo compounds. In the recent decade, several methods have been introduced for removing these compounds from the sewage. Out of all, biological methods have several advantages such as less expenditure, time consuming and environmental friendly.

Methods: In our country one of the main sources of pigmented sewage is texture manufacture. Hence, in order to remove color from this sewage, the present study was conducted to evaluate potential of some bacteria for removing the dye from sewage of texture manufacture in Kermanshah. To perform all this investigates, 30 samples were collected from soil nearby the manufacture and 10 from sewage. Then all bacteria with potential of decolonization were isolated and identified using biochemical tests. The optimization of decolorization for all of bacteria was carried out in second steps with respect to evaluation of bacterial activity at different temperatures (25-45), pHs (5-9) and dye concentrations (0.01-0.04g/l).

Results: The results obtained from this study indicated that 7 isolates had ability to remove dye from the sewage. Out of all, three were gram positive and 4 were gram negative. The gram positive bacteria were belong to genus Bacillus and the gram negative bacteria were belong to Enterobacteriaceae. In addition the results indicated that the best temperature for all isolated bacteria was 35C°, pH was 8.0 and a dye concentration was 0.02 g/l. Furthermore, statistical analysis was performed on all data. The results obtained indicated that all bacteria used in this study had potential for decolroization from 34.6%- 99.4%.

Conclusion: Decolorization of pigmented sewage for elimination of dangerous compounds could be possible using bacteria and in further research could be concentrate on identification of effective genes or enzymes in bioremediation area.

Keywords: Environmental sample, Optimization, Decolorizing bacteria

P716: **Isolation of Photobacterium sp. MV-66 from golf persian**

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Background and Aim: Photobacterium is a genus of gram-negative bacteria in the family Vibrionaceae. Members of the genus are bioluminescent, that is they have the ability to emit light.Many species, including Photobacterium leiognathi and Photobacterium phosphoreum, live in symbiosis with marine organisms. Species such as Photobacterium profundum are adapted for optimal growth in the deep cold seas making it both a psychrophile (an organism capable of growth and reproduction in cold temperatures) and a piezophile (an organism which thrives at high pressures). To isolate marine bacteria with strong bioluminescence in a wide range of NaCl concentration, especially at low salt conditions.

Methods: A luminous bacterium named LuB-1 was isolated from China. It was identified by biochemical analysis and phylogenetic analysis based on the 16S rRNA gene and designated as Photobacterium sp.

Results: The isolate is capable of emitting strong and stable luminescence in a wide range of NaCl concentration from 0·2 to 5% (w/v). For most toxic agents tested in this study, the response of MV-66 was better than that of Microtox™Vibrio fischeri under both low salt (0·9% NaCl) and high salt (2·0% NaCl) conditions.

Conclusion: The strain MV-66 had an obvious predominance of bioluminescence in a wide range of NaCl concentration and better response for heavy metal pollutants and some organic toxicants in both low and high salt toxicity test systems

Keywords: bioluminescence; luminous bacteria; Photobacterium sp.; toxicity assay

P717: **Characterization of bioactive compounds produced by Actinomyces isolated from soil**

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Background and Aim: Nowadays, development of antibiotic-resistant bacteria culminated in demand concerning to find a new source of remedy for treatment of patients to Therefore, the main purpose of this study was isolation and identification of soil origin Actinomyces and characterize their bioactive compounds

Methods: two stains of bioactive producing bacteria were isolated and identified by standard biochemical tests. The bioactive compounds produced by these strains were partially purified, and their activities were evaluated at different pHs, temperatures. Finally antimicrobial spectrums of the compounds were assessed against Staphylococcus aureus, Bacillus cereus, Corynebacterium sp. Listeria monocytogens, Klebsiella pneumonia, Shigella dysentriae , Proteus mirabilis, E. coli and Pseudomonas aeruginosa.

Results: Our results indicated that the best temperature and pH for activity of the bioactive compounds were 37 °C and 7 respectively. In addition, these compounds had an effect on Staphylococcus aureus, Bacillus cereus, Corynebacterium sp. Listeria monocytogens, Klebsiella pneumonia, Shigella dysentriae , Proteus mirabilis. However, Pseudomonas aeruginosa and E.coli were resistant to them.

Conclusion: based on foregoing evidence bioactive compound specially produced by our nativeActinomyces could be considered as an important field for investigation

Keywords: bioactive compounds, Actinomyces, soil

P718: **Extracellular Enzymatic Activity in Halotolerant and Moderately Halophilic Bacteria in Incheh Broun Hypersaline Wetland in Iran**

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Background and Aim: In the last few years, attention to halophilic bacteria has increased. Halophilic enzymes have shown substantially different properties, including their requirements for the high salt concentrations within the range of 1–4M for activity and stability. These enzymes have numerous applications in the industrial production of different items including detergents, foods, pharmaceuticals, leathers, diagnostic reagents and silver recovery. • Incheh Borun hypersaline wetland is located in the north of Iran near the border of Turkmenistan. This wetland is remarkable because of salinity (280g/l) and variation of pH range (2.8 to 6.8).

Methods: • Sampling carried out from soil, water and salt from 4 different sites of wetland in September 2010. Two complex media containing 3 %(SNA medium) and 12 %(HM medium) salt used for isolation. Samples diluted in sterile salt solution 3 % (w/v) and spread on medium which described previously. After four weeks of incubation at 33 ˚ C, different colonies purified with several subcultures. Gram staining, KOH test, Presence of endospore, Motility, Catalase, Oxidase and Nitrate reduction were tested. 55 strains selected randomly for analysis of 16S rRNA and extracellular enzymatic activity .The presence of amylolytic activity on plates was determined qualitatively by following the method described by Amoozegar et al., (2003), using starch agar medium containing 3 % and 12%(w/v) total salt. After incubation at 33 °C for 2 weeks, the plates were flooded with 0.3 % I2–0.6 % KI solution; a clear zone around the growth indicated the hydrolysis of starch. Proteolytic activity determined by method which described by Amoozegar et al., (2008), in skim milk agar containing 10% (w/v) skim milk, 2% (w/v) agar, supplemented with 3% and 12% (w/v) total salt . Clear zones around the growth after 2 weeks were taken as evidence of proteolytic activity. Lipolytic activity detected by growth on medium containing 1% Tween-80 which described by Harrigan et al., (1976). Gelatin-hydrolysis test determined by method which described by Smibert & Krieg (1994), depending on strains requirement, NaCl added to enzymatic medium.

Results: • Totally, 400 strains purified which 194 strains were Gram-positive bacilli, 184 strains were Gram-negative bacilli and 22 strains were Gram-positive cocci. Strains were belonged to the genera Bacillus (18%) ، Marinobacter (15%) ،Halomonas (13%) ،Kocuria (7%) ،Oceanobacillus (7%) ،Dietzia (7%) ،Virgibacillus (7%) ، Chromohalobacter (6%) ،Rhodococcus (4%) ،Micrococcus (4%) ،Paenibacillus (2%) ،Halobacillus (4%) ، Thalassobacillus (2%) ،Arthrobacter (2%) and Desmospora (2%). The main producers of hydrolytic enzymes were Gram-positive bacilli. Ability of 25 strains in protease production was remarkable.Main amylase producers were different species of Bacillus. Gram-positive coccus and Gram-negative bacilli which were identified in this study did not produce amylase.

Conclusion: In Gram-positive bacilli protease was produced by the genera Bacillus, Virgibacillus, Oceanobacillus and Halobacillus. Among Gram-negative bacteria Marinobacter and Halomonas and in Gram-positive coccus, genera Arthrobacter and Micrococcus were protease producers. Strains which belonged to genera Thalassobacillus, Bacillus, Marinobacter, Rhodococcus, Micrococcus, Dietzia and Kocuria had considerable role in lipase production.Different species of Kocuria, Micrococcus, Halomonas, Oceanobacillus and Halobacillus were gelatinase producers.

Keywords: Enzymatic Activity-Moderately Halophilic Bacteria - Halotolerant Bacteria

P719: **Antimicrobial resistance pattern of Escherichia coli isolated from different parts of digestive tract of sheep**

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Background and Aim: The aim of the present study was to determine if differences in resistance patterns of E. coli in different parts of the intestinal tract of sheep are present.

Methods: Intestinal tracts of 24 sheep were sampled at 5 various locations (duodenum, jejunum, ileum, cecum and colon) after slaughter. Samples were cultured on Mac Conkey agar and obtained colonies were confirmed as Escherichia coli based on the biochemical tests results. Isolates were tested for antimicrobial agent susceptibility to 10 antibiotics (colistin, gentamicin, oxytetracycline, trimethoprim-sulfamethoxazole, amoxicillin-clavunic acid, enrofloxacin, ampicillin, cephotaxime, neomycin and florfenicol), using disc diffusion method.

Results: 91.01% (n=81) of isolated E.coli were multidrug resistant and the highest resistance rate was detected for oxytetracycline (93.2%). Based on the results of the binary logistic regression analysis, the percentage of colistin-resistant E. coli isolates was significantly lower in duodenum than in rectum. The percentage of resistance to amoxicillin was significantly higher in rectum in compare with duodenum and jejunum.

Conclusion: In conclusion, antimicrobial resistance pattern of generic Escherichia coli inhabit intestinal tract of sheep depends on localization of sampling and it must be considered in interpreting the results of antimicrobial resistance assay of E.coli isolated from the fecal samples and generalizing results to bacteria colonized in other parts of the digestive tract.

Keywords: sheep; intestinal tract; Escherichia coli; antimicrobial resistance

P720: **"LEVY'S APPROACH" by exploiting VNTR loci, an efficient strategy for genotyping Persian Bacillus anthracis isolates, provided by RVSRI bacterial collection**

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Background and Aim: In 1945, as many as one million sheep succumbed to anthrax in Iran. Despite an operational massive national vaccination scheme against the disease, a number of sporadic and/or epidemic occurrences of anthrax are reported every year affecting human and animal populations. Bacillus anthracis is a Monomorphic Bacteria. Genomic evaluations have indicated consistent nucleotide sequences in more than 99% of their isolates. This uniform structure of genome has placed it in one of the most known clonal bacterial pathogenes in the world. To extend our epidemiological understanding of B. anthracis in Iran, and also to describe the genomic structure, we have employed the Levy's initiative by using VNTR loci to genotype archival and field isolates of B. anthracis deposited at Razi institute.

Methods:: Genomes of the isolates under study were PCR amplicons of three loci including AA03, AJ03 and AT07. The PCR products of 17 B.anthracis isolates were also sequenced to assess accuracy of gel-electrophoresis observation.

Results: The results of this study revealed that the locus AA03 with two alleles each displayed length-polymorphism with 843bp and 931bp, that allele with length 931bp included 7 copy numbers. The locus, AJ03 with two alleles were known throughout lengths of 451bp and 531bp,which respectively contained 3 and 7 copy numbers. In total, 4 different combinational types were detected in the examined isolates among which one was exclusively represented by the Sterne 34F2strain of B.anthracis. This exotic non-pathogenic strain is used for vaccine production with no evolutionary relationship With Iranian population of B.anthracis as observed by the present study.

Conclusion: We believe Levy’s approach can improve the discrimination power of recently standardized 8-loci MLVA genotyping system developed for B.anthracis. It also represents a significant genetic diversity.

Keywords: Bacillus anthracis, genotyping, MLVA,VNTR Levy, Anthrax, AA03, AJ03, AT07

P721: **Comparison of Infectious Bursal Disease (IBD) diagnosis using Colorimetric Gold Nanoparticles and RT-PCR methods**

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Background and Aim: Review: Polymerase chain reaction (PCR) method is used in the diagnostic laboratory, but novel approaches for rapid, simple and specific detection of pathogens is needed in clinical laboratory. The impact of advances in nanotechnology is particularly relevant in biodiagnostics, where nanoparticle-based assays have been developed for specific detection of bioanalytes of clinical interest. Gold nanoparticles (AuNPs) show easily tuned physical properties, including unique optical properties, robustness, and high surface areas, making them ideal candidates for developing biomarker platforms.

Methods: Materials and Methods: In this study, the extracted genomes of 40 unknown samples were conducted to detect for IBD virus using colorimetric gold nanoparticles methods. The obtained results were compared with RT-PCR results of IBD virus.

Results: Results: Our results demonstrate the accuracy of colorimetric gold nanoparticles method with 85.71% and 66.66% sensitivity and specificity, respectively. In most cases the results were overlapped and 24 of 40 unknown samples were detected as IBD viruses with two applied methods.

Conclusion: Conclusion: Detection using PCR is a sensitive and accurate method but it is costly and time consuming method which needs advanced skills and special equipment. Using gold nano-particles with accuracy above 80 %, could be a substitute approach for detection of IBD virus. So this nanoparticle-biomaterial complex could be comprised in the diagnostic kit.

Keywords: Keywords: Gold nanoparticles, PCR, IBD, diagnosis

P722: **Estandardization of pfge techiniqe for genetic analysis of pathogenic leptospiral serovars**

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Background and Aim: Leptospirosis a zoonotic infection caused by pathogenic members of the genus leptospira , is most common in tropical region where incidence peacks during the rainy season.Rats and other rodent are the most common reservoirs,which may transfer infection to domestic animals,and humans.Thus knowledge of the prevalent serovar with genetic characterization and maintance hosts is essential to understanding the epidemiology of this disease. Diagnosis of leptospirosis important for early treatment of patients and for better prognosis.previous studies of human leptospirosis method of identification of isolates has focused on DNA-based technique such as pulsed-field gel elrctrophoresis(PFGE).This technique has been success fully utilized to discriminate between closely related serovars of the leptospira spp. The aim of this study was to genetic analysis leptospira serovars by using PFGE technique.

Methods: All leptospira isolate were used for this study obtained from microbial culture collection microbiology depaetment of leptospira Reference Laboratory Razi Vaccine&Serum Research Institute,Karaj,Iran.Contaning cells and low melting agarose prepared pluges. After lysis with lysis buffer contaning proteinase k,digestion performed by Not I restriction enzyme. Electrophoresis of the prepared samples was performed on a contur-clamped homogenus electric field CHEF DR II sestem.After electrophoresis,the gel was stained sterile solution contaning ethidium bromide and then photographed with gel documentation system.A standard molecular weight marker consisting of concatamers of the lambda ladder was used finally , all fragment patterns were evaluated usually in a dendrogram .

Results: This method determining the pattern of performed with NotI restriction enzyme and resulted in several different patterns that Definite result will be enunciate at the congeress .

Conclusion: Serovar identification is necessary for epidemiological surveillance. In the present study PFGE was standardized and evaluated for its potential utility to rapidly identify pathogenic serovars of leptospira. The result of this study shown that PFGE technique is useful tool for characterization of leptospires in epidemiological purposes. Standardization of PFGE technique for genetic analysis of pathogen leptospiral serovars.

Keywords: Leptospir, PFGE, Fingerpriting

P723: **The prevalence of Escherichia coli O157 in commercial layers in Kermanshah Province**

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Background and Aim: Escherichia coli is a part of the normal intestinal microflora of animals and man. Most strains are harmless, but a limited number of serotypes are responsible for diarrhea or more serious forms of illness. These strains are categorized as enteropathogenic, enterotoxigenic, enteroinvasive, enteroaggregative or enterohemorrhagic according to their pathogenicity. Some strains of E. coli O157 are causes of human infections, especially are capable of producing Shiga toxin. The Shiga toxin-producing Escherichia coli (STEC) of the O157 serotype is the cause of a serious human alimentary infection associated with hemorrhagic or watery diarrhea which may, particularly in children, be complicated by life threatening hemolytic uremic syndrome. The objective of this study was to investigate the prevalence of E. coli O157 in commercial layers in Kermanshah province, west of Iran.

Methods: A number of 170 fresh fecal swab samples, from 5 clinically healthy commercial layer farms in Kermanshah province, were collected and maintained in 5 ml of trypticase soy broth (TSB) containing 0.5 mg ml-1 novobiocin and kept cold while being transported to the laboratory. Each cloacal swab, was incubated at 37°C for 18 hours. Then enriched culture was plated onto eosin methylen blue (EMB) agar. After 24 hours incubation at 37°C, single colonies, with typical E. coli metallic shine on EMB, were streaked onto sorbitol-MacConkey (SMAC) agar plates supplemented with cefixime (0.05 mg ml-1) and potassium tellurite (2.5 mg/L).The inoculated sorbitol-MacConkey (CT-SMAC) plates were then incubated at 37°C for 24 hours. Non sorbitol fermenter (NSF) colonies were selected from CT-SMAC plates and were confirmed as E. coli by biochemical tests. The confirmed sorbitol-negative E. coli colonies were tested with specific antiserum and the positive samples were found to agglutinate.

Results: Out of 170 cloacal swab samples, 55 (32%) were positive for NSF E. coli colonies. E. coli O157 could be isolated from 18 (10.5%) of 170 cloacal swabs.

Conclusion: To our knowledge, this is the first work to reveal the presence of E. coli O157 in commercial layer flocks in Iran. Some strains of this serotype harbor stx1 and stx2 genes. They can play as an important potential source of contamination for people handling and consuming table eggs. In next future the isolates will be tested by molecular methods for possible presence of stx1 and stx2 genes. Further works have to be done on commercial layers and table eggs to examine possible zoonotic pathogens.

Keywords: Escherichia coli O157, shiga toxin, commercial layers, Kermanshah

P724: **Phylogenetic analysis and sequence variations in 16S rRNA gene of Mycoplasma synoviae isolated from commercial chicken farms of Iran**

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Background and Aim: Mycoplasma synoviae (M. synoviae) is an important avian and extracellular pathogen, being responsible for significant economic impact in intensive production, which causes great economic losses in poultry industry.

Methods: To perform phylogenetic analysis and sequence variations of M. synoviae isolates derived from commercial chicken farms of Iran based on 16S rRNA gene, partial sequence of 16S rRNA genes obtained from 19 isolates of central region of Iran (Tehran, Qazvin, Semnan and Markazi provinces) were amplified by PCR in 207bp fragment and then sequenced in order to obtain the genetic variability of strains, after that compared with 16S rRNA gene of M. synoviae sequences which were available in GenBank. Sequences were aligned using ClustalW method (BioEdit 7.0) and Neighbor-joining algorithm (MEGA 5.0) was used to create phylogenetic tree.

Results: Phylogenetic analysis of these sequences showed that all 19 M. synoviae isolated from Iran were most closely related to sequences of M. synoviae from Brazil. Variations, polymorphisms, and differences between nucleotides of all isolates were observed.

Conclusion: The results of this study suggest that the different molecular structure and heterogeneity among M. synoviae isolated may be explained by transmitted the mutations and variations between other countries by reason of high volume of trading or the spontaneous mutations by reason of region conditions. The variations identified in our study are important to better understand the nature and genetic diversity of these microorganisms.

Keywords: Mycoplasma synoviae • 16S rRNA • Phylogenetic analysis • nucleotide variations • Iran

P725: **Comparison between the traditional and bioreactor production of brucella Rev.1 and S19 vaccine in Iran**

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Background and Aim: The aim of this study was to evaluate the brucella vaccine production by traditional Roux flask (solid media)and in fermantor(liquid media) and comparing the yields of final products. The Safety and immunity of both procedures were also studied in animal models.

Methods: Two already used Brucella Rev.1 and S19 vaccine were produced separately in five batches in Roux flask (traditional method) and in fermentor (modified method). The average amount of 4 L of culture were used in both fermentor(5 liters Bio Flo 3000 – Bench top Fermentor M12270052, New Brunswick Scientific, USA)) and Roux flask for the two strains.

Results: The comparison of colony forming unit (CFU/ml) in fermentor showed 4.6 × 1010 for Rev.1 in fermentor and 1.4 × 1011 in Roux flask respectively .The result for S19 strain colony forming unit (CFU/ml) were 1.8 × 1011 and 4.24 × 1011 in fermentor and Roux flask respectively.

Conclusion: Our investigation showed the better bacterial yields production on solid media in Roux flask compared to liquid media in bioreactor for both strains. However, there are some advantages in using bioreactor, e.g. probability of less contamination of culture and decreasing the infection risk to staff and also decreasing the number of labor and cost. Furthermore safety and immunity on laboratory animals showed that vaccine produced the same efficacy both in fermentor and Roux flask. Our results correlated with defined OIE standards. Future work: Optimization of culture media also more field animal study is recommended.

Keywords: Brucella, Rev.1 and S19 strain, Vaccine production,Traditional method, bioreactor production,

P726: **Pathological lesions observed in Chukar Partridge experimentally infected with H9N2 influenza A virus**

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Background and Aim: Influenza A viruses have been isolated from humans and many other mammalian and avian species. Among these viruses, a low pathogenic H9N2 avian influenza A subtype has become panzootic in Eurasia over the last decade. During the past decade, H9N2 low pathogenic avian influenza virus (LPAI) has caused considerable economic loss due to decreased production, increased mortality and the cost of vaccination in the poultry industry of many Asian countries. Because of widespread occurrence of this disease and the virus potential to mutate to highly pathogenic (HP) form and transmission to humans, it is, therefore, imperative to understand the pathogenesis and properties of these viruses. This study was conducted to assess the pathological lesions caused by AIV subtype (H9N2) in different organs of Chukar Partridge.

Methods: A number of fifty 4-month-old Chukar Partridges without any history of previous influenza infection, randomly divided into 5 separate groups (48 Chukar Partridges divided into 4 equal experimental groups and 2 Chukar Partridges were kept as control group). Birds in experimental groups were inoculated with different dose of the virus (group¬0 107.75 EID 50, group¬1 105.75 EID 50, group¬2 103.75 EID 50, group¬3 101.75 EID 50).The clinical signs and gross lesions were recorded. On days 1, 3, 6, 9 and 12 three birds from each group (treatment) were randomly selected and humanly sacrificed. Tissues samples from different organs including the lung, spleen, trachea, thymus, kidney, intestine, cecal tonsil and the pancreas were collected for pathology examination, fixed in 10% neutral buffered formalin solution. Tissue samples were routinely processed to paraffin wax blocks and 5 µm sections were prepared and stained with haematoxylin-eosin (H&E) stain for light microscopic examination.

Results: No clinical signs were observed in different experimental groups. And control group did not show any pathological lesion too. However pathological lesions observed in experimental groups are as follow: the lung showed congestion, hemorrhage, edema and infiltration of lymphocyte on days 6, 9 and12 PI in groups 0, 1 and 3, especially in group 0. In inoculated Chuckar Partridges, lymphocyte depletion in the spleen was seen. Tracheitis associated with deciliation, degeneration of mucous, infiltration of lymphocyte was seen on day 9 PI in group 0. However, on days 6 and 12 PI in group 0 and 1, lymphocytic tracheitis, associated with infiltration of lymphocytes, deciliation were observed. Thymus, cecal tonsil and pancreas showed no depletion of lymphocytes. Pathological lesions were absent in the intestine. Mild hemorrhage and necrosis were observed in the kidney on day 9 PI in group 0.

Conclusion: This study showed that the lung and spleen were affected more severe than other organs. Pathological changes observed in the spleen were more severe than other lymphoid tissues.

Keywords: Pathology, Avian Influenza Virus, H9N2, Chukar Partridges

P727: **Potential effect of Rosmarinus officinalis oil on healing of infected cutaneous wound with candida albicans on rats: a histopathological evaluation**

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Background and Aim: Candida albicans is a diploid fungus that grows both as yeast and filamentous cells and a causal agent of opportunistic oral and genital infections in humans. Candidiasis, caused by Candida species, is a yeast-type fungus that commonly infects the skin and wound in humans; and it is fairly common and can involve almost any area of skin on the body. Beside invasive diseases including candidemia and candidiasis in deep-seated organ, mucocutaneous disorders such as skin and oral candidiasis, vaginal and vulvovaginal candidiasis, have become a problem of significance in clinical practice. Unavailability and expensive drugs, side effects, and particularly, development of drug resistance, led to the use of biological materials to be considered as an alternative solution. Higher and aromatics plants have traditionally been used in folk medicine as well as to extend the shelf life of foods, showing inhibition against bacteria, fungi and yeasts. Rosemary (Rosmarinus officinalis L.), which belongs to mint family, is a common dense, evergreen, aromatic shrub grown in many parts of the world. Essential oils and various extracts of plants have provoked interest as sources of natural products. They have been screened for their potential uses as alternative remedies for the treatment of many infectious diseases. In the present study, we have studied the Rosmarinus officinalis oil effect on healing of infected skin wound with candida albicans on rats.

Methods: In this study on 45 male Wistar-albino rats (weight 200±10 g), after general anesthesia, and an wound square with dimensions 1/5 in the 1/5 cm area between the shoulder, immediately was applied to the wound 0.1 ml of the suspension containing 1/5×107 CFU Candida albicans yeast. Then tested in three groups of 15 rats each (control, topical ointment containing 1.5g and 3g Rosemary oil) were randomly distributed into 5 subgroups of 3 rats each (sample groups on different days) groups. During the project was obtained, the end of days 4th, 8th, 12th, 16th and 20th from wounds of different groups, in order to histopathology and yeast counts by a special punch biopsy specimen.

Results: In the skin excisional wound animal model, Rosemary oil at clinical relevant neither dose (1.5 and 3%) promoted infection wound healing. In the yeast counts evaluation, significant reduces the Candida albicans cloning in treatment groups compared control group ($P < 0.01$). In the histopathology evaluation, neither treatment groups compared control group, especially ointment containing 3g Rosemary oil could significantly reduce the number of poly nuclear cells, increase the mononuclear cells and new vessels formation at day four, and also increase fibroblast cells at day seventh day after wound creation ($p < 0.01$); Although, topical ointment 3% In some cases.

Conclusion: Both dose of Rosemary oil plays a preeminent role in the, anti-fungi and fibroblast-proliferating activities compared control group. Be considered this herbal formula; accelerate infection wound healing and better choice to use a topical ointment containing 3g Rosemary oil.

Keywords: Rosemary oil, ointment, candida albicans, infected wound healing, rats.

P728: **Homological analysis of the ligB gene of L.interrogans dominant vaccinal serovars in Iran**

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Background and Aim: Leptospirosis, caused by infection with pathogenic Leptospira species is the most prevalent zoonotic diseases in the world.The leptospiral vaccines used currently are mainly multivalent dead whole-cell mixtures made of several local dominant serovars. These vaccines, however, do not confer cross-protective immunity and may lead to incomplete, short-term immunity as well as serious side effects. Thereupon design and construction of an efficient recombinant vaccine for leptospirosis control is very important.LigB is an immunogenic protein i.e. expressed only in pathogenic Leptospira spp. Highly conserved LigB antigene is special significance in vaccination and serodiagnosis for leptospirosis.In order to polymorphism analysis we sequenced and compaired ligB genes cloned from pathogenic vaccinal serovars of leptospires prevalent in Iran.

Methods: Three vaccinal serovars of pathogenic L.interrogans (L.Serjoe hardjo,L.Grippotyphosa,L.Canicola)and one saprophytic species (L.biflexa) were used to inoculate into the selective culture medium and extraction of the genomic DNA by standard Phenol-Chlorophorm method. The specific primers for proliferation of ligB gene were designed.The PCR products of pathogenic serovars were ligated in pJET1.2/ blunt vector and transformed in competent E.coli Top10 cells.The extracted recombinant plasmid were sequenced(Macrogen Company, Korea).The percentage of homology and divergence among examined leptospiral serovars was deduced using the MegAlign program of Lasergene software DNASTAR.Homology searches with the ligB sequences of different epidemic Leptospira serovars were accomplished using the BLAST program against sequences in the GenBank/NCBI nucleic acid sequence database.

Results: PCR amplification of the ligB gene using the designed primers resulted in a 1041 bp ligB gene product in all three pathogenic vaccinal serovars tested. No PCR products were amplified from the non-pathogenic L.biflexa. Our results showed that the ligB gene is relatively different among dominant L.interrogans serovars.Minimum sequence identity of the ligB gene was observed between L.Grippotyphosa and L.Canicola (82.6%).while, maximum sequence identity was between L.Canicola and L.Sejroe hardjo (90.1%).

Conclusion: According to the results of this study and other researches,ligB gene nucleotide sequence is different within dominant L.interrogans serovars in Iran.Thus,the differences in nucleotide sequences in the ligB gene types may affect the immunogenicity of LigB proteins. In order to improve efficiency of ligB recombinant vaccine, it can be designed to include all the LigB protein variants.So the cloned gene in this study could be further used for expression and recombinant LigB may be a useful vaccine candidate against leptospirosis.

Keywords: Leptospirosis, Polymorphism analysis, Sequencing, ligB

P729: **Effect of using cow dung and chemical fertilizer on microbial and physicochemical parameters and pathogenic bacteria in cyprinus fish pond water**

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Background and Aim: The aim of this study was comparison the effect of cow dung as organic fertilizer and chemical fertilizer on microbial flora, fish and human pathogenic bacteria and physico-chemical parameters of cyprinus fish pond water.

Methods: The water samples were collected from two ponds, pond 1 was fertilized by cow dung and pond 2 was fertilized by chemical fertilizer, respectively from May to October 2011. . The aerobic and anaerobic bacteria were enumerated in TSA by serial dilution of the sample, followed by conventional pure plate method. Coliforms bacteria were similarly isolated on Chrom agarTM ECC. Moreover, the water samples were used to analysis of BOD, dissolved oxygen, conductivity, transparency, total alkalinity, total dissolved solid, total hardness, total nitrogen and total phosphorous. Temperature and pH were measured by thermometer and portable pH meter in place of ponds. For isolating of fish and human pathogenic bacteria, thirty six fish were collected from pond 1 from July to October and then sampled from their liver and kidney and inoculated in blood agar.

Results: The mean of total count of bacteria and total coliform count in cow dung sample were 4 × 104 ± 1200 and 4700 CFUmL-1 respectively. The mean of water total count bacteria of pond 1 were significantly higher than the water of pond 2. The water total count bacteria of pond 1 were significantly increased in August, but water total count of pond 2 was not shown significant variation from May to October. The results showed that Pesudomonas, Klebsiella, Enterobacter, Yersinia and E.coli were isolated from water of pond 1 but just Pesudomonas and E.coli were isolated from water of pond 2. In this study, the bacteria growths were negative in all of the inoculated plate from fish liver and kidney. The total hardness, total phosphorus, total dissolved solid and conductivity of the pond 1 water were significantly higher than the pond 2 water. But the BOD, dissolved oxygen and transparency of the pond 2 were significantly higher than the pond 1 water. There were no significant differences in the total nitrogen, total alkalinity and pH between water of pond 1 and 2.

Conclusion: In recent years, the chemical fertilizer has been used in cyprinus fish pond but many studies have showed that they were high risked as environmental pollution. Thus, the use of livestock manure especially cow dung could a good alternative. The results showed the use of cow dung increased the bacterial population and diversity. Increasing of bacteria population caused increased total phosphorus that plays an important role in bloom of plankton because we found decreasing transparency and planktons are an important food for cyprinus fish. In despite of isolation of fish and human pathogenic bacteria from water, the bacteria growths were negative in all of the inoculated plate from fish liver and kidney. In summary, it seems the cow dung is a good alternative for fertilizing of cyprinus fish pond water.

Keywords: cow dung, cyprinus fish, microbial and physicochemical parameters, pathogenic bacteria

P730: **Antibiotic resistance in Escherichia coli and Staphylococcus check Avrvyvs isolates from milk of cows with mastitis.**

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Background and Aim: Objective: Mastitis is the most important factor in economic losses in the dairy farming industry in the world. Bacterial species Staphylococcus aureus, a major cause of swelling and general Ashrsha Pathan are Drgav. In order to get control of mastitis and antibiotic resistance detection and successful treatment of resistant strains is important.

Methods: Materials and Methods: In this study 27 strains and 17 Staphylococcus aureus isolates from milk of cows with mastitis Astafylv Kvkvk of Mashhad city dairies were examined. Bacteriological tests for identification of isolates was performed at the Laboratory of Veterinary Medicine, Ferdowsi University of Mashhad. Hasyt isolates tested antimicrobial disk diffusion method using antibiotic disks containing 9: gentamicin and chloramphenicol, and trimethoprim-sulfamethoxazole and cephalothin, cephalexin and Nvrvflvksasyn and Kanamysyn and amoxicillin and ampicillin for E. coli isolates and 8 disks containing antibiotics: gentamicin and chloramphenicol, and trimethoprim-sulfamethoxazole and cephalothin Nvrvflvksasyn and oxy-tetracycline and kanamycin and penicillin for S. aureus isolates was performed.

Results: Results and Discussion: Evaluation of resistance of antibiotics in isolates Ashryshakly resistance to the antibiotics chloramphenicol and oxy-tetracycline and sulfamethoxazole trimethoprim 22/2% and respectively 85/1 of, cephalexin 48/1% and Kanamaysn 29/6% andAmoxicillin and ampicillin was 100%.Jdabh of antibiotics on Staphylococcus aureus to antibiotics sulfamethoxazole and trimethoprim Arytrvmasyn 5/8% and Celine penny 94/1 percent. Overall, 14 E. coli isolates and antibiotic resistance among S. aureus isolates were found in only 2, which shows the variation in antibiotic resistance patterns of multiple resistance in E. coli is the Jdabh.

Conclusion: Conclusion: Indiscriminate and widespread use of antibiotics in the treatment and control of mastitis increases the risk of creating antibiotic-resistant strains. The results showed high rates of antimicrobial resistance among isolates of Escherichia coli bacteria Staphylococcus aureus is Mvldvrm breast. The identification of strains resistant to antimicrobial susceptibility test methods of control measures and treatment are of great importance in the successful treatment of infected cows. However, the transmission of antibiotic-resistant strains to humans through unpasteurized milk is also important in terms of public health.

Keywords: Key words: Escherichia coli and Staphylococcus aureus mastitis and antibiotic resistance.

P731: **Identification and characterization of Mycobacterium abscessus isolated from soil of cattle farms around Shiraz**

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Background and Aim: Mycobacterium abscessus is a rapidly growing Mycobacterium that is a common water contaminant. M. abscessus can cause chronic lung disease, post-traumatic wound infections, and disseminated cutaneous diseases, mostly in patients with suppressed immune systems. The aim of present study was isolation and characterization of mycobacteria from soil of cattle farms around Shiraz. The project is still underway.

Methods: Soil samples from several cattle farms were cultured onto 7H11 plates as well as Lowenstein-Jensen slopes and incubated at 25°C and 37°C until sufficient growth was available.

Results: We managed to amplify the hsp65 gene and analyze it with restriction enzyme digestion. The results showed that the strain is Mycobacterium abscessus. M. abscessus is expected to produce 231 and 210 bp product by BstEII digestion and 160 and 60 bp producet by HaeIII digestion.

Conclusion: The results indicated that different mycobacteria are present in the surrounding environment of domestic animals. They maybe play a role in the resistance to diseases such as tuberculosis and Johne's disease.

Keywords: Mycobacterium abscessus, soil, cattle farms, Iran

P732: **Species level identification of staphylococci isolated from bovine milk samples based on PCR-RFLP analysis of glyceraldehyde-3-phosphate dehydrogenase-encoding gene**

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Background and Aim: Staphylococcal mastitis is a major problem in dairy industry, affecting animal health and causing economic losses throughout the world. The aim of this study was to identify the most common staphylococcal pathogens involved in bovine cases of clinical and sub-clinical mastitis using PCR-restriction fragment length polymorphism (RFLP) analysis of gap gene (933 bp), which encodes the glyceraldehyde-3-phosphate dehydrogenase.

Methods: From the 158 mastitic milk samples collected from 7 dairy herds located in 4 different areas of East and West Azerbaijan provinces, Iran, 113 staphylococci were isolated. AluI digestion of gap PCR-generated products rendered distinctive restriction patterns that allowed 10 Staphylococcus spp. to be identified.

Results: Overall, Staphylococcus haemolyticus (40.7%) and Staphylococcus chromogenes (15.7%) were the most common coagulase-negative staphylococci (CoNS) species found followed by S. epidermidis, S. warneri, S. cohnii (10.2% each), S. simulans (5.5%), S. hominis (3.7%), S. capitis (2.7%) and S. xylosus (0.9%). S. haemolyticus, S. chromogenes and S. warneri were the only species identified from clinical mastitis. The distribution of some Staphylococcus species was herd-specific.

Conclusion: The present study indicated that CoNS were the most common bovine mastitis isolates and could therefore be described as emerging mastitis pathogens in the North West of Iran. To our knowledge, this was the first study providing genotypic characterization of staphylococci isolated from bovine milk samples in Iran.

Keywords: staphylococcal mastitis, bovine, gap gene, Iran

P733: **Genetic characterization of penicillin and methicillin resistance in staphylococci from bovine mastitis milk in the North West of Iran**

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Background and Aim: The objective of this study was to investigate the genes implicated in resistance to penicillin (blaZ) and methicillin (mecA) among staphylococci isolates recovered from bovine mastitis using PCR method.

Methods: A total of 113 Staphylococcus spp. was detected in 158 mastitic milk samples (71.5%) by conventional methods and by genus-specific PCR. The isolates were then identified to the species level using gap PCR-RFLP.

Results: The species identified were S. haemolyticus (40.7%), S. chromogenes (15.7%), S. epidermidis, S. warneri, S. cohnii (10.2% each), S. simulans (5.5%), S. hominis (3.7%), S. capitis (2.7%) and S. xylosus (0.9%). In the study, 74 of the 113 staphylococcal isolates were determined by PCR to be blaZ gene positive (65.5%), whilst the mecA gene was detected only in three coagulase-negative staphylococci (CNS) including S. hominis (n=2) and S. epidermidis (n=1).

Conclusion: The findings of this survey indicate that the prevalence of methicillin-resistant staphylococci carriage is low for bovines with mastitis, but that carriage of penicillin-resistant staphylococci is considerably higher. Moreover, bovine mastitic milk could be a reservoir of blaZ- and mecA-positive staphylococci, with potential implications in public health

Keywords: Staphylococci, bovine mastitis, blaZ, mecA, Iran

P734: **Molecular typing of nasal carriage isolates of Staphylococcus aureus from healthy animals based on multiple locus variable number tandem repeat analysis (MLVA)**

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Background and Aim: Staphylococcus aureus is an opportunistic pathogen commonly found as part of the normal flora of the nares. The aim of the study was to determine the molecular diversity in S. aureus isolates collected from nasal cavity of healthy animals housed at different geographic areas, Iran.

Methods: A total of 42 Staphylococcus aureus isolates were recovered from horses (n=18), cattle (n=4), sheep (n=12) and goats (n=8). Multiple locus variable number tandem repeat analysis (MLVA) was used for molecular typing.

Results: According to the results, 12 different profiles were identified (A-L). Twenty six (62%) of the isolates belonged to either type A or B. However, types A and B were not detected in goats and sheep, respectively. Types C, D, K, and L (all together accounting for 16.7% of all isolates) were only detected among S. aureus isolates from horses. Types G and H were restricted to sheep isolates and type F was only found among goats isolates. Type E contained isolates from horse and sheep, type I from horse and cattle, and type J from sheep and goats.

Conclusion: In conclusion, MLVA analysis revealed a large diversity of genetic content among the analysed S. aureus nasal isolates and identified some genotypes as a frequent colonizer.

Keywords: Staphylococcus aureus, nasal, healthy animals, MLVA, Iran

P735: **Isolation and Molecular Identification of lactic acid producing bacteria from the rumen of Sanjabi sheep**

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Background and Aim: The rumen is a complex ecosystem where billions of bacteria, archaea, protozoa and fungi reside. This diverse microbiota is well adapted to live in the rumen and play an important role in the digestion of feeds and nutrients supply to the host animal in the form of microbial protein and volatile fatty acids.This study was conducted to isolate and identify lactic acid producing bacteria (LAB) in the rumen of Sajabi sheep.

Methods: The rumen contents were obtained from three rumen fistulated animal fed on mixed ration (alfalfa hay and concentrate (4: 1)) twice a day. Forthy-eight lactic acid producing bacterial isolates (excluding streptococci) were cultured from the rumen contents on the m-BA medium and Basal Medium 10/ Roll tubes. After total DNA was extracted, 16S rDNA was amplified by PCR from the genomic DNA of each isolate. The primers were 27f (50-AGAGTTTGATCCTGGCTCAG-30) and 1492r (50-TACGGYTACCTTGTTACGACTT-30). Following, RFLP analysis with the restriction enzymes ALU1 and Dde1, the PCR products of the 10 isolates were subjected to near complete 16S rDNA sequence analysis.

Results: DNA sequencing results designated 99% similarity with Enterococcus hirae and 86% resemblance with Enterococcus faecium.

Conclusion: An acidic environment favors the rapid proliferation of LAB due to acetic condition of the rumen, resulting in increased lactic acid and a further decline in the pH. Therefore, identification and characterization of LAB from the rumen should bring about to our knowledge and controlling of fermentative acidosis.

Keywords: lactic acid producing bacteria, Enterococcus hirae, Enterococcus faecium, acidosis

P736: **Use of Probiotics in Aquaculture - a review**

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Background and Aim: The term probiotics was first used by Lilly & Stillwell in 1965. Probiotic was defined as the microbiological origin factor that stimulates the growth of other organisms. In 1989 Roy Fuller introduced the idea that probiotics generate a beneficial effect to the host. He defined probiotics as live microorganisms which, when administered in adequate amounts, confer benefit to the host's health, improving the balance of the microbiota in the intestine.

Methods: Probiotics are defined by Food and Agriculture Organization/World Health Organization as “live microorganisms which when administered in adequate amounts confer a health benefit on the host”

Results: The purpose of its use is to install, improve or compensate for the functions of the indigenous microbiota that inhabit the digestive tract or the surface of the body. The idea of using fermented foods for some health benefits is not new, being mentioned in the Persian version of the Old Testament (Genesis 18: 8) that “Abraham attributed his longevity to the consumption of sour milk”. Later, in 76 BC, a Roman historian, Pline, recommended the use of fermented milk products for the treatment of gastroenteritis cases

Conclusion: The use of growth promoters allows improving the zootechnical performance of animals. Initially a large variety of substances with antibiotic function was used to improve performance of poultry, pigs and cattle, especially penicillin and tetracycline. The use of antibiotics as additives to feeds showed great benefits to animal husbandry, expressed primarily in improved weight gain and feed conversion.

Keywords: Probiotics, Aquaculture

P737: **Stability studies on the vaccine diluent used at Razi vaccine and serum research institute**

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Background and Aim: In house prepared physiological saline is used for diluting the lyophilized animal vaccines manufactured at Razi institute. This diluent is prepared by WFI made at Razi institute and sodium chloride salts. This study was conducted in order to determine the stability of the mentioned diluent.

Methods: Pyrogeny, sterility and Physico-chemical tests were performed on three different batches of the diluent stored at variable temperature for a period of three years.

Results: According to results the diluent was stable between 4 to 37°C during the tested time period.All samples appeared sterile, non pyrogenic and no change in their pH and sodium chloride content was recorded during the tested time period.All results were in acoordance with the British pharmacopia 2011.

Conclusion: To conclude, the expiry date of the diluent as mentioned on the label is two years at cool temperature (below 15C) but based on the results it is suggested that the expiry date of this product could be extended to three years at room temperature.

Keywords: vaccine diluent, pyrogeny, stability, sterility.

P738: **Antibody response and growth performance of Broiler chickens fed Bacillus subtilis as native probiotic**

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Background and Aim: Intestinal microflora plays an important role in maintaining the balanced of immune response against infectious agents. probiotics such as microflora increase resistance to a variety of pathogens microorganisms. The aim of this study was to investigate the effects of probiotic bacteria, contain Bacillus subtilis isolated from the intestines of chickens, on the induction of antibody responses in chickens vaccinated with infectious bursal fabricius virus as well as its effects on chicken's body's weight.

Methods: 1-day-old broiler chickens (cobb 500strain) were randomly grouped and each groups received the following compounds: group 1: Control without vaccine, group 2, control with vaccine, group 3: probiotic and vaccine (1ml with 1×109 cfu/ml). Vaccine was given orally on days 17 and 26. The rate of total antibody was determind with ELISA test, after each week bleeding. Body weight was also weekly determined.

Results: primary titers of antibody was similar in all groups (5257± 1071 u / ml) and significantly were decreased d until day 25 (p < 0 /05). For example, in Groups 1, 2 and 3 the titers of antibodies was 1428±162/6, 640/7±137/8 and 717/3±56/26, respectively. In this day declining of antibody titers in groups 2 and 3 were significantly higher and had significant difference with control group. In group1, decreasing of antibody titers was continued until day 32 (968/2±141/4 u / ml) while in groups 2 and 3, the titers was increased and were up to 2654±226/2 and 3638±218. u/ml respectively. At the end of day 32 in group that received probiotic, titers of antibody in compared to the other groups were increased significantly. Our results showed that administrating of probiotic was significantly enhanced broiler performance by improving body weight. (1622 ± 56.88 gr).

Conclusion: The use of Bacillus subtilis as native probiotic can stimulate the immune system and increase the titers of antibody, Hence it will increase the resistance in chickens and make them more resistant to infectious bursal disease. Administrated Bacillus subtilis as probiotic could enhance the broiler performance and decrease food conversion ratio (FCR) which is the main factor affecting the profit of broiler industry.

Keywords: probiotic, bursa of fabricius virus, antibody, immune system

P739: **evaluation of cytopathic effect of E.coli O157: H7 on vero cell**

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Background and Aim: Shiga toxin-producing Escherichia coli (STEC) serotype O157: H7 has emerged as one of the most important causes of foodborne infections worldwide.Most enterohemorrhagic E.coli strain intimately adhere to vero cell line in a characteristic cytopathic effect pattern ,which is mediated by bacterial shiga toxin. Shiga toxins (Stx1 and Stx2) are the major virulence factors of these strains.Stx is a member of the A-B5 family of toxins, which are composed of one enzymatic A subunit, noncovalently bound to a B subunit composed of a homopentamer of B fragments. Cells that have receptor (Gb3) are sensitive to cytotoxic effects of toxin. It has been shown that various tumor cells have Gb3 receptor and are selectively sensitive to apoptotic effect of verotoxin. The aim of this study was to comparison of cytotoxic effect of verotoxin 1 and 2 of E.coli O157: H7 strains on Vero cell line (gold standard for evaluation of cytotoxic effect of Verotoxin).

Methods: In this research 150 samples consisting of recto-anal mucosal swabs were collected from cattle and sheep in Razi Research institute shiraz.Samples were detected as E.coli by enrichment, biochemical and Serological test, then PCR assay were used to detecte E.coli O157: H7.They were checked for the presence of the stx1 and stx2 gene using multiplex-PCR.The toxigenic strain was cultured .the vero cells monolayer were performed in 12-well.After incubation ,pure stx1, stx2 and both of them ,and also Ecoli O157: H7 bacterial culture re-added to each well , Vero cell was treated with serial dilutions of toxin .After 24 h of exposure and incubated at 37 degree C and 5% CO2,the result of cytopathic effect (CPE) were evaluated by inverted microscope.

Results: E.coli O157: H7 were detected in 16(10/66%) of the 150 recto-anal mucosal swabs samples .Multiplex PCR showed that 1 (6/25%) isolates carried stx2 genes , 8 (50%) both stx1 and stx2 and 7 (43/75%) whitout stx1 and stx2.Our result indicated that Verotoxin has cytotoxic effect on Vero cell lines. This effect is directly related to toxin concentration and incubation time.

Conclusion: Our results revealed that Verotoxin of E.coli O157: H7 strains has cytotoxicity on Vero cells and this effect on Vero cells is Depends on kind of toxin ,Stx-2 has been shown more effective than Stx-1 or both of them.The Vero cell assay, in particular, is a good model to evaluate of cytopathic effects of pathogen bacteria.

Keywords: E.coli O157: H7 , Stx-1, Stx-2, vero cell

P740: **Isolation of Corynebacterium xerosis from native egg shells**

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Background and Aim: The microbiological safety of foods, especially animal products is an increasing public health concern worldwide. During a study conducted to investigate the contamination of native chicken eggs in Kermanshah province to aerobic bacteria, Corynebacterium xerosis could be isolated from two egg shells. Although Corynebacterium species occur commonly in nature in the soil, water, plants, and food products, Isolation of Corynebacterium xerosis from chicken egg shells has not been reported before.

Methods: Total number of 200 native chicken eggs were collected from five locations of Kermanshah province, placed in sterile plastic bags and transported to laboratory. Eggs shell contaminations were determined by using floating the egg in peptone water, and surface culture on plate count agar. Plates were incubated at 37°C for 24 hours. Preliminary identification of the clinical isolates as Corynebacterium spp. was performed following standard procedures. After incubation; typical or suspicious colonies were examined and further confirmed by culture on blood agar and Gram stain, microscopic and biochemical tests.

Results: Based on distinctive features for the identification of Corynebacterium xerosis such as: Gram-positive, catalase positive, non spore-forming, non motile, rod-shaped bacteria that are straight or slightly curved and biochemical tests, isolation of Corynebacterium xerosis from two eggs shells was confirmed.

Conclusion: Chicken eggs can be contaminated by microorganisms via two major routes, vertical and horizontal. Vertical transmission (transovarian infection) occurs before the egg covered with the shell. Horizontal transmission includes trans shell infection of the contents of the egg during transit through the cloaca or after oviposition. Corynebacterium xerosis could not be isolated from egg white or egg yolk. Thus, isolation of such bacteria from chicken egg shell might be as the result of contamination through handling by human. The report of the identification of Corynebacterium xerosis from clinical samples or products of animals may be useful for veterinary clinical microbiologists to improve the knowledge of its distribution and its possible association with disease in animals and man. Corynebacterium xerosis is a normal commensal of human skin and mucous membranes which can cause dermatitis, bacteremia, endocarditis, mediastinitis and pneumonia in immunosuppressed patients. Further studies have to be done to done to reveal the molecular characteristics of isolates.

Keywords: native chicken egg, Corynebacterium xerosis, Kermanshah

P741: **Burden of Rotavirus in Diarrheal Newborn Calves in Tehran and Alborz Provinces, Iran, during 2010-2012**

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Background and Aim: Group A rotavirus (RVA) is the most common cause of severe gastroenteritis associated with significant morbidity, mortality, and economic burden among children and newborn calves worldwide. Determining the rate of rotaviruses in calves' diarrhea is a key issue for deciding of the development and the introduction of effective vaccines. Among the conventional techniques for diagnosis of rotaviruses, ELISA and RT-PCR are most using methods which rely on rotaviral antigens and genes, respectively, for showing of presence of rotaviruses in diarrheal stools. The purpose of this study was to estimate the prevalence of RVA strains among newborn diarrheic calves in dairy farms in Tehran and Alborz provinces, Iran, in a two-year surveillance and also to investigate of real prevalence of RVA by gene based methods in comparison to antigen based methods.

Methods: From March 2010 through March 2012, 253 stool samples of diarrheic calves up to 4 weeks old were collected from 42 industrial dairy farms located in two Iranian provinces. For investigation of involvement of rotavirus group A in calves' diarrhea, first, commercially rotavirus ELISA test was done by using supernatant of the stool samples. Also, after extraction of the nucleic acids from the stool samples, RVA was detected by a one-step RT-PCR targeting the VP6 coding gene. Finally, positive samples were analyzed by excel software and seasonally distribution of rotavirus in diarrheic calves was assessed.

Results: Globally, RVA was detected in 96/253 (37.9%) samples and 148/253 (58.5%) samples, respectively, by ELISA and RT-PCR. Also, prevalence of RVA infections fluctuated according to the seasons with a peak of infection in the autumn and winter seasons (67.2% and 80.4%, respectively) when temperatures were low, against spring and summer (40.5% and 53.6%, respectively). Although the mentioned rates are from RT-PCR methods, ELISA also showed the same profile for seasonal distribution of rotavirus infections.

Conclusion: This study is the first study reporting the prevalence of RVA strains circulating in diarrheic calves of one of the greatest husbandry centers in Iran by RT-PCR. Furthermore, the results of this study showed prevalence of RVA in cold seasons are more than warm seasons maybe due to resistance of the virus in cold condition. Epidemiological knowledge of involving of rotaviruses in gastroenteritis is critical for proving the importance of rotaviruses in calves' diarrhea. So, these data could help to make informed decisions as to whether rotavirus vaccine should be considered for inclusion in cattle immunization program in Iran.

Keywords: Rotavirus, Gastroenteritis, Bovine, Epidemiology, RT-PCR, ELISA

P742: **Comprehensive and multi aspect analysis of phylogeny, Recombination rate and similarity of Iranian strain of Crimean-congo hemorrhagic fever (CCHFV)**

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Background and Aim: Crimean-Congo Hemorrhagic Fever (CCHF) is a viral disease characterized by hemorrhage and fever. It is a severe disease with a high mortality rate (50% in Iran). Iran is one of the main victims of this disease. Ticks carry the virus from animal to animal and from animal to human. The geographical distribution of the virus, like that of the tick that carries it, is widespread The CCHF virus infects a wide range of domestic and wild animals that serve as reservoirs for the virus. Thus is it essential to study this virus from different aspects.

Methods: At first nucleotide sequences of M & S segments belonging to Iranian strain of CCHF virus retrieved from Databases. After alignment and edition, sequences are examined for similarity of Iranian strains to each other, conserve and variable regions along sequences. Also M and S segments tested for similarity with other virus in different geographical regions, specially neighbor's countries. At next steps probability recombination in M & S segment were analyzed. Finally sequences directed to phylogenic tree by neighbor joining and overall mean distance was estimated.

Results: After Alignment it was clear that M segment has high Conservation among sequences in Iranian strains of virus (only five variable sites seen) and S segment could be to some extent conserve and variability was significant. Similarity evaluation of S & M segments showed Afghanistan, Oman, Tajikistan, Turkey, Uzbekistan and China had nearest similarity with Iranian strains, respectively. Results of phylogenic tree showed 2 main branches and 5 distinct sub branches for S segment, and 2 main branches and 4 distinct sub branches for M segment. Overall mean distances for S and M segments were 0.086 and 0.006 respectively. Analysis revealed significant recombination in Iranian strains with each other and also with neighbor countries in S segment.

Conclusion: CCHFV is placed in Category C by the National Institute for Allergy and Infectious Diseases due its ability to cause outbreaks with high mortality and its association with nosocomial infections. Thus, the development of control measures has priority for public health systems. Viral sequence comparisons among isolates may provide valuable information regarding the molecular basis of the epidemic potential of the virus. Here, we analyzed Iranian strains and its phylogenetic relationship and recombination prbability to other strains of CCHFV.

Keywords: Crimean-congo hemorrhagic fever (CCHFV), phylogeny, Recombination rate, similarity

P743: **Isolation and serological characterization of sorbitol-negative Escherichia coli recovered from urban pigeons in Kermanshah province**

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Background and Aim: Pigeons are distributed in public areas and are potential reservoirs for pathogenic bacteria. Shedding pigeon feces in urban environments may contribute to spread of pathogenic agents. These might include human pathogens such as the shiga toxin-producing E. coli strains, which are able to survive under adverse environmental conditions for extended periods of time. These pathogens may enter the human food chain and cause serious health problems. As pigeons may act as reservoirs or carriers of shiga toxin-producing strains of E. coli and because of capability of some serotypes of sorbitol-negative E. coli, especially E. coli O157, to produce shiga toxins, this study was conducted to isolate and characterize sorbitol-negative E.coli in urban pigeons (Columba livia domestica) in Kermanshah province.

Methods: Total number of 130 fresh fecal samples from urban pigeons (Columba livia domestica) in Kermanshah province, were collected and maintained in 5 ml of trypticase soy broth (TSB) containing 0.5 mg ml-1novobiocin and kept cold while being transported to the laboratory. Each sample was incubated at 37°C for 18 hours. Then the cultures were plated onto eosin methylen blue (EMB) agar. After 24 hours incubation at 37°C, single colonies, with typical E. coli metallic shine on EMB agar, were streaked onto sorbitol-MacConkey (SMAC) agar plates supplemented with cefixime (0.05 mg ml-1) and potassium tellurite (2.5 mg/L). The inoculated sorbitol-MacConkey (CT- SMAC) plates were then incubated at 37°C for 24 hours. Non sorbitol fermenter (NSF) colonies were selected from CT-SMAC plates and were identified as E. coli by biochemical tests. Using a commercial kit, the sorbitol-negative E. coli colonies were tested with specific antiserum for E. coli O157. The test procedure and analysis of results were performed as recommended by Kit manufacturer.

Results: Sorbitol-negative E. coli could be isolated from 20 fecal samples (15/38%). Out of 20 sorbitol-negative E. coli, 7 samples (35.0%) were confirmed positive as E. coli O157.

Conclusion: Regarding the free-flying birds, there are limited number of surveys investigating the prevalence and characteristics of E. coli in the pigeons. To our knowledge, this is the first work to reveal the presence of E. coli O157 in urban pigeons in western parts of Iran. Some strains of this serotype harbor stx1 and stx2 genes. Urban pigeons can play as an important potential source of contamination for people directly and indirectly in contact with them. In next future the isolates will be tested by molecular methods for possible presence of stx1 and stx2 genes. Further works have to be done on pigeons to examine possible prevalence of zoonotic pathogens in other parts of Iran.

Keywords: Escherichia coli O157, pigeons, Kermanshah, sorbitol negative

P744: **Effects of the bacterium Escherichia coli Enterotoxigenic**

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Background and Aim: Entrotoxigenic Escherichia coli (ETEC) infection is one of the most common type of calves colibacillosis. Addition the most common cause of diarrhea among travelers and children in developing countries . White diarrhea or calf diarrhea is a major cause of mortality be with calf. Acute and highly fatal disease of the newborn calves that mortality a few hours to 3 weeks after birth, the calf is suffering., According to studies white calf diarrheal disease be created before the industrial farms much attention is not. With the advancement of technology, creation of industrial farms transfer transport pregnant animals can cause disease. Since the government and the farms imports of foreign cattle and pregnant cows imported into the country, the disease has manifested. The main virulence attributes to ETEC are adhesions K99 and F41 fimberia and neurotoxins specially heat stable toxin.

Methods: Using tubes and rectal swab samples were obtained from calves with diarrhea. A total of 30 samples from the dairy industry, 15 out of 15 samples of semi-industrial dairy cattle husbandry in the traditional province and a total of 60 samples were prepared. Samples with less than 24 hours in ice and transported to the laboratory and were analyzed with the usual methods of E.coli bacteria. That first line cultured on MacConkey agar and EMB agar was prepared and the lactose-negative colonies were suspected E.coli IMViC reaction. Biochemistry of E.coli bacteria were detected in nutrient broth in the presence 20% glycerol at - 70 ° C were stored until needed.

Results: In this study, 60 of the 30 cases of diarrhea in calves, cattle, industrial, semi-industrial farms, 15 of 15 patients were tested traditional Azgavdary. Ten of the 60 isolates from cases of diarrhea in 16.6%. Detected.All bacteria isolated ETEC virulence genes were PCR Some consist mainly of three virulence genes were only two. Fact that the study was obtained from the majority of cattle in traditional calves with diarrhea, respectively. Fact that the diarrhea of a was isolated from the dairy industry

Conclusion: Diarrhea syndrome of the most important diseases of calves in the first month of life, and diarrhea caused by E. coli is the most common cases of diarrhea in the first week of life. The ETEC strains are the most common seven-day live calves

Keywords:: ETCT, White diarrhea ,ST

P745: **Helminthic parasites fauna of Digestive system and liver of sheep in Hamedan province, Iran, in year 2010**

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Background and Aim: The aim of present survey was determination of digestive system helminthic parasites and liver parasites of sheep from cities of Hamedan Province in year 2010.

Methods: Alimentary canals (from oesophagus to rectum) were examined. Oesophagus was surveyed with direct observation. Other parts of digestive canal were examined by washing their contents separately. The worms found in the washed contents of alimentary canal and liver were collected, counted and identified microscopically after being cleaved in lactophenol. also, Abomasum, small and large intestine were examined and information were recorded.

Results: Ostertagia had the most infection which about 71.8 % and the least infection belonged to cooperia with about 0.3 %. Also Results show that 92.98% livers were intact whereas 3.41%, 1.35%, 1.19% were infected by Fasciola spp., Hydatic cyst and Dicrocoelium spp., Respectively. Parasite contamination survey of liver in Hamedan and its cities shows that the Fasciola contamination in spring, summer and fall was more than Hidatid cyst and Dicrocoelium contamination, while in winter, Dicrocoelium contamination was the most.

Conclusion: By attention to above lines we could conclude that Epidemiology strategies could control and prevent of digestive-parasitic disease in sheep by using of many methods such as destroy host, prevent grazing in contaminated regions, change pasture by summer-quarters and winter-quarters, grazing periodically, prevent contamination of food materials and ect.

Keywords: Epidemiology, digestive system and liver parasites, sheep, Hamedan Province

P746: **Using of SrRNA 16 primer for detection of Mycoplasma agalactiae**

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Background and Aim: Mycoplasmas are the smallest free-living microorganisms found so far and consists of wall-less prokaryotes which have unusually small genomes. The mycoplasma cause great economic losses, since they are widely distributed and many of them are pathogenic in humans, animals, and plants. Furthermore, they often contaminate cell cultures. The main factors for identification are culture and polymerase chain reaction(PCR) methods. Mycoplasma agalactiae(M. agalactiae) is considered to be the classic agent of contagious agalactiae, which occur worldwide. Aim of this study, isolation and identification of M. agalactiae from lesions of the eye, ear and joint fluid of goats were suspicious.

Methods: The samples included(conjunctiva swabs, synovial fluid and ear canal swabs) were examined by cultural and PCR methods. The minced tissue were inoculated to PPLO broth agar. After multiple passages, typical mycoplasma colony was isolated. DNA was extracted from enriched samples. Two primers (forward and reverse) amplify a 163bp region of 16S rRNA gene of Mycoplasma genus and two primers amplify 375bp region of 16S rRNA gene of M. agalactiae species were used.

Results: The genus Mycoplasma in culture of samples was 12.5%. As of the genus Mycoplasma in eye, ear swabs and joint fluid of sampling goat were 26.4%, 16.6% and 20%, respectively. To confirm the presence of Mycoplasma genus, gene was amplified by using PCR. The SrRNA 16 primer was bands specific gender with bp163 and FS2 primer was ability to identify species by amplified fragment of the gene Lipoprotein with bp375. All positive genus Mycoplasma samples were made to isolate M. agalactiae.

Conclusion: Positive samples caused by M. agalactiae was 5.7%. Results show that other species of M. agalactiae can exist.

Keywords: mycoplasma, mycoplasma agalactiae, culture, polymerase chain reaction.

P747: **Determination toxicity of epsilon toxin of Clostridium perfringens type D in two culture mediums**

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Background and Aim: Clostridium perfringens is one of the most pathogenic species in the Clostridium genus as it is able to produce at least 17 toxins. The Clostridium perfringens epsilon toxin is one of the most potent bacterial toxins that accountable create many illnesses in human and animals. Aim of this study, determination of toxicity of epsilon toxin of Clostridium perfringens type D in enrichment and enrichment adding with liver powder culture mediums.

Methods: Clostridium perfringens type D cultivated on basis enrichment medium for provision final seed. Culture media for cultivation of final seed include enrichment media(routine media) and add commercial liver powder (7gr/lit) to half of mediums. After assurance of decontamination, final seed inoculate for eighteen hour. Before accomplishment step detoxification, collected samples transfer to laboratory. Minimum lethal dose(MLD) assay carried out as in vivo test for determining of toxicity of epsilon toxin of Clostridium perfringens type D test.

Results: The results get of MLD test in tow groups have 4000 and 4333, respectively. Amount of evaluation total protein are not attribute significant difference in experimental groups.

Conclusion: An the other hand, define that to added commercial liver powder to medium Clostridium perfringens type D in nonfermantory system no effect in toxicity of Clostridium perfringens Type D.

Keywords: clostridium perfringens, culture, liver powder, toxicity, MLD test.

P748: **Assessing of expressed recombinant protein Bm86 by using GAVAC vaccine**

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Background and Aim: After mosquitoes, ticks are known as the most important vector of disease-causing pathogens in humans, domestic and wild animals. Economic losses due to tick infestation estimated at billions of dollars worldwide. They produce damage whether directly by their host blood feeding and indirectly by transmission of different infectious agents. To control tick infestation, there are a variety of methods among which chemical control using Acaricides are widely used. However, application of Acaricides may leads to environmental pollutions and emerging of resistance strains. The other control strategy is immunological methods by using vaccine against ticks. Nowadays, there are two types of commercial vaccines, TickGARD (Australian) and GAVAC (Cuban) which contain a protective molecule of 89KDa glycoprotein which known as Bm86. In the present study to evaluate recently expressed recombinant Bm86 molecule in Razi Institute, we were immunized Balb/C mice using the Cuban GAVAC vaccine and then evaluate the immune response of mice by indirect ELISA using recombinant Bm86 protein as coated antigen.

Methods: Three adult females Balb/c mice were immunized by the Cuban GAVAC vaccine by complete immunization schedule. Three stages of multi-site subcutaneous injection followed by final Intra-peritoneal injection in two weeks' time interval were used. Blood was collected 2 weeks after the final injection and tested in indirect ELISA. The indirect ELISA was developed g/ml of expressed recombinant Bm86, blocking by 1% BSAμby coating of 1 solution and testing with obtained immunized mice serum.

Results: Positive response by increase OD of samples displayed possible similarity between recombinant Bm86 expressed in Razi institute and Bm86 in Cuban GAVAC vaccine.

Conclusion: Taken together, this study indicates that there are similarity between recombinant Bm86 expressed in Razi institute and available Bm86 in Cuban GAVAC vaccine so we can use the recombinant Bm86 to producing a new vaccine.

Keywords: GAVAC,Tick GARD,Bm86

P749: **Ameri-Ziaei Double Antibiotic Synergy Test (AZ-DAST), a novel evaluation method of antibiotic combination**

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Background and Aim: Chemicals considered as the one of the first and most important remedy in the infectious diseases. Concurrent use of two or more antimicrobial agents is common clinical practice for patients with deteriorated disease. The inadequacy of variety of assays to recognize antimicrobial interactions is one of the most important factors to non-use of laboratory methods for selecting antimicrobial combinations. In the current study we introduce AZDAT, a novel double antibiotic synergy test for this purpose.

Methods: Two antibiotic combinations have been evaluated by the AZDAST and compared with the disc approximation technique as a reference method. The antibiotic combinations included penicillin G plus gentamicin and erythromycin plus clindamycin which were tested on Staphylococcus aureus ATCC 25923 and Escherichia coli ATCC 25922.

Results: Based on the average of the growth inhibition zones, using a combinations of penicillin G and gentamicin, AZDAST demonstrated a stronger effect against both bacteria, when compared to using each antibiotic individually (increased 2.19% and 2.90%, respectively). However, a combination of erythromycin and clindamycin using AZDAST demonstrated a weaker or opposite effect against both bacteria (decreased 3.42% and 14.42%, respectively).

Conclusion: The results of this study show that AZDAST can be used as a reliable test instead of the traditional disk approximation technique; however more studies are required to compare this methodology with other assays.

Keywords: Ameri-Ziaei AZDAST Antibiotic Synergy Combination

P750: **The Effect of Silver Nanoparticles and Beta-Lactam Antibiotics individually and in Combination Together on Staphylococcus aureus Isolated from Cattle Mastitis**

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Background and Aim: Antibiotic resistance in Staphylococcus aureus strains against beta-lactam antibiotics have specified. Because of drug resistance the treatment of staphylococcal mastitis is difficult and resistance transmission to human is possible. Use of modern technology for treatment is necessary and the therapeutic properties of silver nanoparticles have already been proven. Nanoparticles in nanomedicine are defined as particulate dispersions or solid particles with a size in the range of 10-100 nm. Silver nanoparticles (AgNPs) show ample antibacterial activities, which confer to them a major advantage for the development of alternative products.

Methods: In the present study, 311 milk samples were collected frommastitis casase in cattle. California mastitis test (CMT) used for detection of mastitis cases and bacteriological cultures carried out on all milk samples in order to identify the Staphylococcus aureus. In addition to accurate identification of Staphylococcus aureus, nuc gene was amplified by PCR. The minimum inhibitory concentration (MIC) and the minimum bactericide concentration (MBC) of silver nanoparticles and antibiotics individually and in combination were determined on 50 Staphylococcus aureus isolate.

Results: Patterns of Staphylococcus aurous isolates resistant to penicillin, amoxicillin, ampicillin and cefazolin, were 50%, 56%, 46% and 30% respectively. 8% of all isolates were sensitive to low levels of silver nanoparticles. In all of the isolates, cefazolin in combination with nanoparticles had synergistic effect.

Conclusion: Based on this study, silver nanoparticles in combination with beta-lactam antibiotics have more effect on Staphylococcus aureus isolates than any of the antibiotics or silver nanoparticles alone. These findings support use of the AgNPs in combination with Beta-lactam antibiotics as effective medicine of mastitis infected with Staphylococcus aureus.

Keywords: Antibiotic resistance, Staphylococcus aureus, Antibiotics affecting on cellwall, Mastitis, Silver nanoparticles

P751: **Characterization of isolated salmonella by PCR-RFLP shiraz**

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Background and Aim: Salmonellosis is one of the most common food-borne bacterial disease s in the world that is one of the major public health problem which salmonella causes in disease. In most food animal species, Salmonella can establish a clinically in apparent infection of variable duration, which is significant as a potential zoonosis. The genus Salmonella consist of over 2668 different serotypes. These microorganisms cause disease in both humans and animals.. The most common sources of Salmonella include beef, poultry and eggs,. Dairy products, vegetables, fruits and shellfish have also been implicated as sources of Salmonella. . Salmonella also is one of the most important causes of gastroenteritis in human and poultry are mentioned as a zoonotic reservoir for this agent. Infections with bacteria of the genus salmonella are responsible for a variety of acute and chronic diseases in poultry documented. The aim of this study, identification salmonella isolated from poultry in shiraz by polymerase chain reaction restriction fragment length polymorphism(PCR_RFLP).

Methods: 31 isolated of Salmonella DNA was extracted using the phenol- chloroform method and used as template in the PCR and With using special primers (Fsa-R and Fsa-F) were amplified for detection of fliC gene, and 1500bp fliC fragment was amplified from all of serovars. After that, PCR products were digested by HhaI Endonucleases with using of the 100bp DNA ladder was as the molecular weight marker in the RFLP analysis..

Results: 31 isolated of Salmonella DNA was extracted using the phenol- chloroform method and used as template in the PCR and With using special primers (Fsa-R and Fsa-F) were amplified for detection of fliC gene, and 1500bp fliC fragment was amplified from all of serovars. After that, PCR products were digested by HhaI Endonucleases with using of the 100bp DNA ladder was as the molecular weight marker in the RFLP analysis..

Conclusion: Results of PCR-RFLP showed that this molecular technique has a directand so These surveys revealed that PCR-RFLP method based on fliC gene was a simple method with a high discriminatory power and it can be used to determine Salmonella serotypes and the PCR- RFLP analysis was unable to differentiate all the serovars salmonella.

Keywords: Salmonella ,fliC gene , PCR-RFLP, poultry, HhaI , shiraz

P752: **Isolation, identification and antimicrobial susceptibility of clostridium clostridioforme isolates from Broiler chickens**

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Background and Aim: Clostridium bacteria are gram-positive, anaerobic, and spore forming. Clostridium clostridioforme is unique in several respects. It typically stains Gram negative, the bacilli are often found in pairs with tapered ends, and spores are difficult to find. The C. clostridioforme group comprises three principal species (C. clostridioforme, C. bolteae, and C. hathewayi) that differ in virulence and antimicrobial susceptibility despite similar colony and microscopic morphology. C. clostridioforme is a phenotypically heterogeneous anaerobe that has been involved in a variety of infections, including bacteremia. The purposes of the current collaborative study were isolation, identification and evaluate the susceptibility of Broiler chickens C. clostridioforme isolates to antimicrobial agents.

Methods: One hundred-twenty feces samples from the gastrointestinal tract of chickens from different poultry house of Kerman province were randomly selected. After preparation and cultivation, gram staining of pure colonies was done. The biochemical tests as described in Bergey's manual were carried out to identify the C. clostridioforme. Fifteen different antimicrobials were selected for antimicrobial susceptibility testing using the Kirby-Bauer disc diffusion technique on Muller Hinton agar using the readymade antibiotic discs. The antimicrobial agents that were tested and their concentrations (µg or unit) were as follows: penicillin G (10), oxacillin (1), ampicillin (10), amoxicillin (25), cephalothin (30), chloramphenicol (30), tetracycline (30), clindamycin (2), vancomycin (30), ciprofloxacin (5), bacitracin (0.04), streptomycin (10), erythromycin (15), gentamicin (10), and trimethoprim + sulfamethoxazole (1.25/23.75).

Results: From 79 Clostridium isolates, thirteen isolates (16.45%) was identified as Clostridium clostridioforme. Results showed susceptibility to Cephalothin, Chloramphenicol (84.62%), Ampicillin and Amoxicillin (76.92%). Vancomycin, Ciprofloxacin and Trimethoprim + Sulfamethoxazole (46.15%) and Gentamicin (53.85%) showed intermediate susceptibility. In this study, the resistance to antibacterial agents was found to be widespread among the Clostridium clostridioforme isolates. The most frequent resistance was observed to Bacitracin (90.91%), and then to penicillin G and Streptomycin (both 84.62%), and Erythromycin (76.92%). Resistance of bacteria to Tetracycline, Oxacillin and Clindamycin was 69.2%.

Conclusion: Isolation of C. clostridioforme from broiler chicken illustrated the importance of this type of bacteria in poultry. Resistance to penicillin G probably is due to β-lactamase production. The multiple and variable resistance patterns observed in this study among the C. clostridioforme group isolates, demonstrate the challenge faced by veterinarians in the field in choosing the correct antibiotic. Thus, it is important for microbiology laboratories to distinguish between C. clostridioforme group species and for clinicians to be aware of differences between them. We would suggest that further researches in antibiotics susceptibility and resistance of C. clostridioforme.

Keywords: clostridium clostridioforme, biochemical test, Antimicrobial susceptibility

P753: **Antimicrobial resistance among clostridium leptum isolated from Broiler chickens**

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Background and Aim: Microbiota from the cecum of broiler chicken is very diverse. One of The predominant cecal bacterial groups is Clostridium leptum. This cluster plays a key role in inhibiting the establishment of intestinal pathogens such as Salmonella enterica. Also, C. leptum subgroup is predominant in the colonic microbiota of healthy humans and that's one of the most predominant populations in the human fecal microflora. This subgroup includes bacteria that produce butyrate, a source of energy for intestinal epithelial cells. The metabolic activities of these organisms have a significant effect on the health of the human colon. The use of antibiotic growth promotants in poultry rearing is a public health concern due to antibiotic resistance in bacteria and the harborage of resistance genes. To improve our understanding about the C. leptum, evaluation of the resistance of Broiler chickens C. leptum isolates to antimicrobial agents was performed.

Methods: One hundred-twenty feces samples from the cecum of chickens from different poultry house of Kerman province were randomly selected. After preparation and cultivation samples, gram staining of pure colonies was done. The biochemical tests as described in Bergey's manual were carried out to identify the C. leptum strains. Fifteen different antimicrobials were selected for antimicrobial susceptibility testing. The disk diffusion method was performed as described by the National Committee for Clinical Laboratory Standards (NCCLS). The antimicrobial agents that were tested and their concentrations (µg or unit) were as follows: penicillin G (10), oxacillin (1), ampicillin (10), amoxicillin (25), cephalothin (30), chloramphenicol (30), tetracycline (30), clindamycin (2), vancomycin (30), ciprofloxacin (5), bacitracin (0.04), streptomycin (10), erythromycin (15), gentamicin (10), and trimethoprim + sulfamethoxazole (1.25/23.75).

Results: From 79 Clostridium isolates, nine isolates (11.39%) was identified as Clostridium leptum. C. leptum have showed Resistance to Cephalothin, Bacitracin, Penicillin G, Oxacillin, Streptomycin, Tetracycline, Erythromycin, Ciprofloxacin and Trimethoprim + Sulfamethoxazole. Chloramphenicol (55.5%), Gentamicin and Clindamycin (44.4%) showed intermediate susceptibility. Results showed susceptibility to Vancomycin (100%), Ampicillin (77.7%) and Amoxicillin (66.6%).

Conclusion: We found that C. leptum dominated the cecal environment. Isolation of C. leptum from broiler chicken illustrated the importance of this type of bacteria in poultry. Basis of our results C. leptum is resistance to many of Antibiotics. Thus it can grow readily in gastrointestinal tracts of broiler chicken and inhibiting the establishment of intestinal pathogens. We would suggest that further researches in antibiotics resistance of C. leptum.

Keywords: Clostridium leptum, biochemical test, Antimicrobial resistance, Broiler chickens

P754: **Serological studies of Newcastle disease and avian influenza in slaughter-age poultry birds in some area of kerman province**

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Background and Aim: Avian Influenza (AIV) and Newcastle Disease Viruses (NDV) are two important culprit in poultry diseases causing serious economic loss in the poultry industry in the world as well as in Kerman. Therefore, the present study was carried out to determine a serological study on Avian Influenza and Newcastle Disease Viruses in poultry in the selected areas in kerman.

Methods: The blood samples were collected randomly from 441 broilers of 15 flocks in kerman province between September and October 2012. broilers were sampled in last week of growing period. Sera were prepared by centrifugation at 1800 x g for 10 minutes and transferred to sterile microtubes kept at 4◦C until the moment of use. Serum samples were examined for detection of anti AI and ND antibodies by using of commercially available ELISA kits (AIV and NDV Anti-body Test Kit, BioChek, Gouda, The Netherlands). The ELISA test s′and analysis of results was performed according to the manufacture recommendations.

Results: Overall seropositivity of AIV and NDV in birds was determained 49/2% and 88.1%, respectively.

Conclusion: the ELISA test is very easy and rapid, less laborious, less time consuming for the detection of AIV and NDV. Although ELISA is a good choice for testing a large number of serum samples, this procedure cannot distinguish between ARV vaccinated and infected serum samples .In summary, although both disease shows seropositivity but the rate of AIV positive samples is higher than NDV in kerman province. Authors suggest that because of these infections may result significant economic losses in poultry industry it needs more researches in this region.

Keywords: ELISA, AIV, NDV, Kerman

P755: **A comparison between bacterial culture and PCR in identification of American foulbrood (AFB) and European foulbrood (EFB)**

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Background and Aim: American foulbrood (AFB) and European foulbrood (EFB) are important bacterial diseases in honeybee that have worldwide distribution and are the lethal potential agent in larva of honeybee in infected colony. The agents of diseases are fastidious and diagnosis with conventional method and biochemical diagnostic kit are time consuming. The aim of this study is comparison to conventional method and molecular method (PCR) in identification of AFB and EFB.

Methods: In present study larves with clinical symptoms have examined for disease using conventional methods and PCR simultaneously. Two professional primers with were specific to 16S rRNA gen.

Results: The result of this study showed that the number of positive samples was 13.3% in PCR but 10% with conventional method such as bacterial culture for Paenibacillus larvae. All samples in both bacterial culture and PCR method were negative as infection to Mellisocuccos plutonius

Conclusion: Finally, it is strongly recommencement to use molecular method such as PCR for identification of AFB. As EFB agent could not be identified is this study so the investigation of disease in other province, using conventional and molecular method is necessary.

Keywords: Honey bee, American foulbrood, European foulbrood, PCR

P756: **Examine and identify bacteria in the digestive tract of poultry broiler chickens at different ages Ilam**

Zohre ARVANEH[1], Zohre Arvaneh[2], Mahtab Abdi[3], khalil saleki[4]

1- Zohre Arvaneh
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3- Scholar
4- tutor

Background and Aim: as while as digestive is important as organg of growth and health and also meet and other production chickens is one of main sources protein for human. And also a strong link between animal and human diseases, the control of livestock diseases is very important. Therefore, in this study the identification of intestinal bacteria in poultry broiler chickens Elam was targeted.

Methods: 114 cases of live broiler chickens of different ages (1 to 38 days) poultry Ilam laboratory of Microbiology, University of Ilam in sterile portion of the duodenum, ileum and cecum and colon poultry samples were taken after the contents are provided for the Rich in nutrient agar medium and selenite - F at 37 ° C for 24 h and cultured for detection of bacteria of the biochemical tests (fermentable sugar, glucose, lactose, producing gas SH2; reaction of indole, the use of citrate and reaction methyl Red - move Vgazprvskvr test was used.

Results: In this study, bacteria was isolated from stool samples was different with statistics obtaiend in other investigations. in a report that Carried out from slaughterhouses East Tehran in 1373, the prevalence of Salmonella in poultry carcasses from 5/75%, Yousefi Mashoof in research that has don in Hamadan City has reported the frequncy of salmonella to 6/8 per cent in 1379. Creman City of the Salmonella group, 5/12% of the bacteria isolated from the intestines of chickens were conducted in Hamadan is consistent with the study. After all tests as mentioned abuve on sampels most isolated bacteria were identified in each city. one day old chicks in the city of Ilam 62.5% of Klebsiella, Enterobacter Sirwan in 25%, 17-day-old chicks in Dareshar City Enterobacter 66.66% rate and 35-day-old chicks Ilam City75% Citrobacter most isolated. Ilam 31-day-old chicks were also equally (50%) and Proteus and Mvrganla, and also 2 Days old chicks was isolated only in Proteus in Dereshahr City.

Conclusion: Based on the results of biochemical tests performed on 114 samples tested intestinal bacteria, most of the bacteria are Enterobacter, Proteus and Klebsiella.

Keywords: poultry, stool culture, purification

P757: **Urinary tract infection caused by Streptococcus spp. as a naturally occurring disease in laboratory hamsters (Mesocricetus auratus)**

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Background and Aim: Most animals currently maintained in conventional laboratory animal settings are kept under optimal sanitary situations which eliminate the potential hazard of transmission of common zoonotic agents. However, random sampling is regularly done in conventional animal houses for evaluating the prevalence of naturally occurring zoonotic and non-zoonotic disease which cause potential health hazard for researchers or limit the usage of animals in biomedical researches. This clinical report, represent the urinary tract infection caused by Streptococcus spp as a common disease in laboratory hamsters which has not been reported later.

Methods: In Kerman medical school conventional laboratory house, each season 30 golden hamsters (10% of overall hamster population) in three equal group (preweaning, 3-9 month and 9-18 month) were sacrificed for evaluating the prevalence of naturally occurring disease. This survey was done during July to December 2012. Necropsy was done for all animals and proper samples were obtained for bacteriological, parasitological and histopathological evaluations.

Results: Among 60 sacrificed animals, the most prevalent disease were intestinal parasitism by Syphacia muris (38%), followed by urinary tract infection caused by streptococcus spp. (30%), wet tail disease caused by Escherichia coli (18%) , amyloidosis (8%) and infestation with Rodentolepis nana in 5.5% of overall population which only the last one is zoonotic. Syphacia muris and Rodentolepis nana infestation, amyloidosis and urinary tract infection were most common in 9-18 month group, whereas wet tail were more common in preweaning group. Urinary tract infection and amyloidosis were interestingly more common in female compare to male hamsters but the prevalence of other disease was not related to gender.

Conclusion: Although the Syrian (golden) hamster, Mesocricetus auratus, is relatively new to biological research, it is the third most commonly used laboratory animal in many countries. When compared to the mouse and rat, information is limited on the incidence, relative importance, and occurrence of spontaneous diseases of the Syrian hamsters. Bacterial diarrhea are among the most important and well-described diseases of the Syrian hamsters. Salmonellosis, Proliferative ileitis and typhlitis caused by campylobacter coli and clostridial infections by Clostridium perfringens and C. difficile are common gastrointestinal diseases in hamsters however amyloidosis is the only common reported disease in the urinary tract of hamsters. Group B streptococcus is reported as a leading cause of urinary tract infection in human medicine but to the best of author's knowledge, this is the first report of urinary tract infection caused by Streptococcus spp in hamsters. It seems that asymptomatic bacteriuria could cause epizootic urinary infection in laboratory hamsters. Further investigation must be done for serogroupig of isolated bacteria for clarification of zoonotic potential's of these organisms. Reporting of similar cases could yield useful information about the occurrence of spontaneous diseases in laboratory hamster. Since the presence of intercurrent disease can seriously jeopardize experimental results, information of this nature would be potentially valuable to investigators.

Keywords: Urinary tract infection, Laboratory hamsters, Streptococcus spp.

P758: **Elisa for detection of specific IgG In Bovine Leptospirosis**

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Background and Aim: Leptospirosis is an infectious disease with a worldwide distribution that is caused by pathogenic spirochetes belonging to the genus leptospira .laboratory confirmation is essential to reach an accurate diagnosis.the gold-standard microscopic agglutination test(MAT) requires the maintenance and handlingof live cultures of leptospires,which is tedious and time-consuming but enzyme-linked immunosorbent has been found to be a more sensitive serological test than the conventional methods for diagnosis of leptospirosis.

Methods: Antigen was prepared from a well grown culture of leptospires(109 ml-1)in EMJH medium.100µl of diluted serum reacts with leptospiral antigens attached to the polystyrene surface of the microwell ,the plate was incubated at 37• for 1 hour and washed five times with buffer.conjugated antibody was then added and mixed well then read at a wave length of 450 nm.

Results: On testing different samples of sera from infected and non-infected animals, the ELISA exhibited sensitivity and specifity of 80% and 87% respectively.

Conclusion: the elisa method is suitable for large numbers of serum samples and it needs skill of examination,laboratory equipment and experinced personnel,elisa could serve as a good choice for early and rapid diagnosis of bovine leptospirosis

Keywords: ELISA,MAT,BOVINE,Leptospira

P759: **Antifungal activity of nano-essence Artemisia sieberi against M. canis isolated from feline dermatophytosis**

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Background and Aim: Dermatophytoses are superficial infections of keratinized tissue caused by dermatophytes. Microsporum canis is responsible for most dermatophytoses in cats, its natural host, and dogs. Due to limited number of antifungal drugs are available against dermatophytes species and there is an urgent need to identify new efficient antifungal targets, the objective of this research was to determine the susceptibility of M. canis to nano-essence Artemisia sieberi by Broth Microdilution. Artemisia sieberi grows wild in different regions of Iran and grows in desert and semi- desert climate and has forage value for animals and also medicinal properties.

Methods: The antifungal activities of nano-essence Artemisia sieberi essential oil were evaluated against M. canis reference strain (PTCC) and10 clinical isolates. In vitro susceptibilities were determined as described in CLSI document M 38-A2. Microdilution minimal inhibitory concentration (MICs) ranges following 48 h s of incubation were 0.03–2µl/ml for nano-Artemisia sieberi essential oil. MICs were determined subculture and by visual inspection comparing the growth in the 96-wells containing the drug with the nano essential oil free control.

Results: The antifungal susceptibility analysis showed that all the strains of M. canis analyzed were sensitive to nano-Artemisia siberi (0.06 µl/mL ? minimum inhibitory concentration (MIC) ? 0.1µl/mL). MICs obtained by the microdilution method showed no significant differences between isolates in this study. Compare to previous studies, nano-essence Artemisia sieberi was more effective than Artemisia sieberi.

Conclusion: The antifungal properties of this nano essential oil showed good potential use in against M. canis infection. We conclude that effective antifungal activity of nano-essence Artemisia sieberi under study, against M.canis isolated from feline dermatophytoses and this nano-herbal drug is good replacement treatments for cutaneous fungal infections such as dematophytoses.

Keywords: antifungal susceptibility, Microsporum canis, minimal inhibitory concentration, nano artemisia sieberi

P760: **Seasonal variation of milk somatic cell counts in Qazvin milk collecting centers**

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Background and Aim: Somatic cell count (SCC) is an indicator of the quality of milk.(SCC) is composed of leukocytes or white blood cells, that are produced by the cow's immune system in response to inflammation in the mastitis. The number of somatic cells increases in response to pathogenic bacteria like Staphylococcus aureus, a cause of mastitis. The aims of this study were to describe associations of season with cow somatic cell count (SCC) in qazvin milk collecting centers.

Methods: 102 samples of 28 milk collecting centers were selected. Milk samples were analyzed for (SCC) using a DeLaval Cell Counter.

Results: the results showed that (SCC) in qazvin milk collecting centers was highest in summer and winter, and ranged up to 500,000 cells/mL in 30 percent of samples. But in autumn and spring only 5 percent of the sample ranged up to 500,000 cells/mL.

Conclusion: Results here indicate that (SCC) varied widely during the year according to season. Considering the economic losses due to high (SCC) and the increase in the risk of mastitis, the establishment of management procedures to control the hygiene of cows is recommended to reduce losses.

Keywords: Somatic cell count, milk, milk collecting center

P761: **Influence of Calendula officinalis alcoholic extract on in vitro phagocytosis activity in cattle**

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Background and Aim: Many studies certify the antiviral, antibacterial, antifungal activity of various plant extractions such as Calendula officinalis and the immunostimulating activity of many vegetal compounds has been described.The purpose of this study was to determine the in vitro activity of alcoholic extract of Calendula officinalis as potential stimulators of the innate cell-mediated response in cattle.

Methods: Blood samples collected from non-pregnant dairy Holstein cows (aged: 2-4 years; n=8), which were treated in vitro with alcoholic extract of Calendula officinalis (5 and 10 µl), using the 70° alcohol as a control. In vitro evaluation of phagocytosis activity in blood samples was performed using the carbon particle clearance test in two periods i.e. 0-20 and 20- 50 min.

Results: According to the results, during the first period (0-20 min) of testing, the Calendula officinalis extract had inhibiting effects compared to the alcohol control. For the second period (20 - 50 min) of reading, the extraction acted stimulating when used 5 and 10 µl of the Calendula officinalis extract.

Conclusion: In conclusion, it was concluded that the alcoholic extract of Calendula officinalis has immunostimulating activity in cow blood samples and it may has more beneficial effect against the most bacterial infections in dairy cows.

Keywords: Calendula officinalis, phagocytosis, in vitro, cow.

P762: **Ideutifiication of salmonella isolated from karaj by PCR- RFLP**

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Background and Aim: Salmonella is a genus of Gram-negative rod-shaped bacteria of the family Enterobacteriaceae. Infection with Salmonella in humans and animals primarily causes self-limiting gastrointestinal infections with mild to moderate symptoms, including fever, abdominal cramps, and diarrhea. More severe clinical outcomes, including death, may occur in cases of bacteremia or enteric fever (typhoid), which is often characterized by severe headaches and high fever but no diarrhea They cause a wide range of human diseases such as enteric fever, gastroenteritis and bacteremia.In general Poultry and poultry products, in particular chicken meat and eggs, are considered important sources of human infection with this pathogen. Salmonella can also be found on fresh produce, including tomatoes and on dry foods such as pet food. All salmonella serotypes potentially are pathogens, so can contaminate various animals. Salmonellosis is a food-borne disease that is among one of the major public health problems.Salmonella infection arising form contaminated food continues to be an immense problem with millions of cases occurring throughout the world. The aim of this study was to isolate Salmonella from poultry and identification by using fliC gene and polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) analysis.

Methods: 1- Salmonella spp. from Razi Type Culture Collection, Microbiology Dept, Razi Vaccine & Serum Resaerch Inst. 2- Serotyping of isolated salmonella 3- DNA extraction 4- PCR With using special primers (Fsa-R and Fsa-F), for detection of fliC gene, (1500bp) 5- PCR-RFLP by HhaI Endonucleases

Results: The present study was followed to identify of salmonella isolation from poultry in Karaj.A total of 30 salmonella identified by Biochemical tests and serotype with antiserum, which includes , S. Enteritidis(3/3%), S. uno(33/3 %), S.dorban(53/3 %), S. tinda(3/3%) S. strenbos(3/3%) , S. ninstedton(3/3%) , S. new port(3/3%) , S. mijimweme(3/3%) , S. tampsom (3/3%) ,S.SIIO8(3/3 %),S.SIIO7(3/3%), was following by PCR- RFLP , showed 5 pattern between 30 isolated.

Conclusion: Results of PCR-RFLP showed that this molecular technique has a directand so These surveys revealed that PCR-RFLP method based on fliC gene was a simple method with a high discriminatory power and it can be used to determine Salmonella serotypes

Keywords: Salmonella , Flic gen , Polymerase chain reaction restriction fragment length polymorphism, poultry , humans, HhaI,

P763: **Survey on Helminthes Infection in Domestic Geese of Jouybar, Mazandaran, Iran**

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Background and Aim: Domesticated birds, including domestic geese have grown in most parts of the country. Weed and waste residues of agriculture production be used for the food consumption of geese. Geese have become adapted to live with warm and cold weather. Contact birds with soil to obtain nutrients and water, cause contamination to different types of parasites which could cause significant economic losses. Also breeding with other domestic birds in their natural environments, could be exchanged these parasites between them. Therefore, knowledge about parasitic infections of these birds, particularly helminthes infection and its role in parasitic infection of poultry are essential.

Methods: During 5 months, 20 gooses gastrointestinal were collected from villages around the city of Jouybar randomly. The samples were placed into formalin and were transported to the laboratory. Different parts of the digestive tract including the esophagus, crop, proventriculus, gizzard, small intestine and cecum was separated and then was opened longitudinally. The contents were well washed and the mucosa was completely scraped and contents were evaluated for the worm by the Stereo microscope. In order to precise diagnosis, worms were clarified by Lactophenol then these were evaluated under the microscope with the lenses 10 and 40.

Results: Five sample (25 %) had been helminthes infection totally. Prevalence of Amidostomum anseris and Heterakis gallinarum was 15%, 10% respectively. Although in normal conditions, the clinical symptoms of parasitic infestations isn't very high but even in this condition, presence of parasites could cause subclinical form of parasitic infection. These birds may be encountered with laying down and weight loss. The symptoms of the disease (coused by A. anseris) are in keeping with the character of the tissue alterations brought about by the activity of the parasites in their usual habitat, particularly underneath the cuticle of the gizzard. Mild infestation with A.anseris may occur without the manifestation of disease, such infested birds would serve as carriers of the parasite. In this study, all isolated worms were the nematode. These worms due to their direct evolution are able to survive throughout the country. The amount of isolated A.anseris is more than other worms that it is similar to results of other researches.

Conclusion: Given the importance of goose as significant food source, particularly in north area of Iran, dealing with pathogenic agents (especially parasites) that could threaten their health and production, is essential.

Keywords: Domestic Geese, Amidostomum anseris, Heterakis gallinarum, Jouybar

P764: **Prevalence of gastrointestinal helminthes of local chickens, in northeast of Iran**

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Background and Aim: Local chickens prevail in rural areas where they play an important role in the livelihood of smallholders and in the agricultural practice. In addition, local chickens are a source of meat and eggs for rural families. Several constraints face local chickens and their production systems. Helminthosis is one of the most common diseases that affect scavenging chickens. Free-range scavenging chickens are in direct contact with parasite vectors, soil and feces. On the other hand, inadequate hygiene and the physical environment (e.g. rainfall, humidity, and ambient temperature) provide optimum conditions to maintain helminthes populations. We conducted a cross-sectional study from December 2010 to August 2011. The study aimed to determine the prevalence of gastrointestinal helminthes among local chickens in Khorasan Razavi province in northeast of Iran.

Methods: A total of 100 male and female local scavenging chickens were selected randomly. We examined the gastrointestinal tract of each bird for the presence of helminthes. The gastrointestinal tract was opened in a longitudinal section; the mucosa was scraped in order to collect the helminths embedded in the mucosal layer. All helminths collected were fixed and identified. Nematodes were preserved in 70% ethanol; cestodes were transferred to acetic formalin alcohol container; stained with acetocarmine and then mounted in Canada balsam. The helminth species were identified by morphological characters according to soulsby

Results: Three nematode and two cestode species were diagnosed. No trematodes were found. Seventy two birds (72%) were infected. The prevalences of different species were as follows: Ascaridia galli 29%, Heterakis gallinarum 23%; Heterakis isolonch 9%,Subulora brompti 3% ; Choanotaenia infundibulum 4%, Raillietina echinobothrida 11%; and Raillietina tetragona 15%.

Conclusion: Finding of the present study indicate that the majority of scavenging chickens in the country are parasitized throughout the year with two or more species of gastrointestinal helminthes and thus may be a cause of tremendous economic loss to Iranian chicken farmers.

Keywords: Gastrointestinal helminthes, Chicken, Khorasan Razavi, Iran

P765: **Development of an ELISA system for detection of epsilon toxin**

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Background and Aim: Enterotoxaemia is a fatal enteric disease that affects all species of domestic animals and is caused by type D of Clostridium perfringens . This bacterium is grouped into five types (types A, B, C, D and E) according to the four major lethal toxins, alpha, beta, epsilon, and iota (α, β, ε, ι) produced.. Epsilon toxin produced by C. perfringens type D is the cause of enterotoxemia in sheep and probably in goats. Immunity against enterotoxemia in sheep is readily produced by vaccination with toxoids. The potency testing of Clostridium perfringens type D enterotoxaemia vaccine is currently performed with mouse neutralization test (MNT) to estimate the levels of epsilon antitoxin in the sera of sheep immunized with enterotoxaemia vaccine. The in vitro serological method like indirect ELISA has been developed for the determination of specific antibodies against epsilon toxin of Clostridium perfringens in the sera of sheep. In this study we set an indirect ELISA system for determination of antibody levels in rabbits were received vaccine on several occasions.

Methods: Three group of rabbit were considered as: vaccinated, none vaccinated (as control) and vaccinated using an expired date vaccine (presumably with low levels of potency). Vaccinated animal received 2 ml of vaccine on 0, 21st day subcutaneously. Blood sample were taken from all animals, 14 days after second vaccination. Sera were collected from the samples and kept at -20 ºC until use. An epsilon toxin pure preparation was used as antigen for coating plates in order to set up an ELISA system. Antibody levels against epsilon toxin in animal sera were assessed by the ELISA system.

Results: Our results showed that vaccination was able to raise antibodies against epsilon toxin and detected properly by ELISA system.

Conclusion: ELISA system can be further improved in to a standard kit for measurement of anti epsilon toxin antibodies in order to assess potency of vaccine. Detection of anti epsilon antibodies can also use in assessment of immune status of herds and animal exposures to the disease agent.

Keywords: elisa, epsilon, vaccine

P766: **In vivo antifungal effect of topical Mentha piperita essential oil ointment on infected skin wound (Tissue yeast count)**

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Background and Aim: Candida is a genus of yeasts. Many species are harmless commensals or endosymbiosis of hosts including humans, but other species including Candida albicans, can cause disease such as mucosal and cutaneous disorders such as oral and skin candidiasis, vaginal and vulvovaginal candidiasis, candidemia. Hence unavailability and expensive drugs, side effects, and particularly, development of drug resistance, led to the use of biological materials to be considered as an alternative solution. One of these biological materials, medicinal herbs is Mentha piperita. Mentha species are used for their flavoring and medicinal properties widely throughout the world. It widely used in food, cosmetics and medicines, distributed in the southwest of India, China, Iran and other countries. Mentha extract and essential oil has traditionally been used to treat various diseases of breath, procreation and digestive systems in the countries mentioned. Primary investigation on this plant has led to the isolation of polyphenolic acids, several flavonoids and mono terpenoids which caused anti-bacterial and anti-fungal effect. Mentha piperita L. is a medicinally important plant that belongs to the family Labiate that grows in Iran. The aim of the present study was to investigate the in vivo antifungal effect of topical Mentha piperita essential oil ointment in differential doses, on infected skin wound with candida albicans on rats, through yeast count of the tissue samples.

Methods: In this study on 45 male Wistar-albino rats (weight 200±10 g), after general anesthesia, and an wound square with dimensions 1/5 in the 1/5 cm area between the shoulder, immediately was applied to the wound 0.1 ml of the suspension containing 1/5×107 CFU Candida albicans yeast. Then tested in three groups of 15 rats each (control, topical ointment containing 1.5g and 3g Mentha piperita essential oil) were randomly distributed into 5 subgroups of 3 rats each (sample groups on different days) groups. During the project was obtained, the end of days 4th, 8th, 12th and 16th from wounds of different groups, in order to histopathology and yeast counts by a special punch biopsy specimen.

Results: In the skin excisional wound animal model, Mentha piperita essential oil at clinical relevant neither dose (1.5 and 3%) accelerate infection wound healing. In the yeast counts evaluation, the number of Candida albicans colonies on the fourth day, in the treatment group 3% versus 16.33×106 ± 15.27 ×105, and treatment group 1.5% versus 27.33×107 ± 14.32×106, compared control group 50.33×107 ± 2.5× 107 ($P < 0.0001$); and on the sixteenth day, the amount of was significant reduced to 32.33×103 ± 16.01×103 in the treatment group 3%, 23.33×104 ± 15.27×103 in the treatment group 1.5% and compared 29.00×104 ± 26.45× 103 control group($P < 0.0001$).

Conclusion: Both dose of Mentha piperita essential oil can cause a number of Candida albicans colonies compared control group. Be considered this herbal formula and our result, dose 3%, have stronger effects than dose 1.5% and can reduce the number of yeast in granulation tissue, thereby accelerating infection wound healing.

Keywords: Mentha piperita essential oil, ointment, candida albicans, infected wound healing, rats.

P767: **Combination of Single Nucleotide Polymorphism(SNP) and Multiple-Locus Variable-Number Tandem Repeat(MLVA) typing methods, for subtyping of Salmonella enterica serovar Enteritidis**

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Background and Aim: Salmonella enterica serovar Enteritidis(S.Enteritidis) is the leading etiological agent of salmonellosis in human and poultry. To understand the genetic diversity of S.Enteritidis isolates from different regions of Iran we used a combinational strategy, exploiting Single Nucleotide Polymorphism(SNP) as an added value approach to Multiple-Locus Variable-Number Tandem Repeat(MLVA). It's been reported previously that, when the matter of evlolutionary relationtionships of isolates in homogeneous pathogenic clones is raised, SNPs are placed among the most valuable molecular markers.

Methods: We Performed MLVA and SNP on 69 chicken isolates from 18 broiler farms and six non-epidemic human isolates totally from six distant geographically regions of Iran. Seven standard loci namely, SE2, SE3,SE5, SE7, SE8, SENTR4 and SENTR7, reported as the most diverse loci were used for the MLVA part of this study combined with a domestically-designed Salent18 locus by Tandem repeat finder software(TRF) which were exploited later in SNP assessment All findings were reconfirmed with sequencing of PCR products. A standard Enteritidis strain(ATCC13076) was also included in our study.

Results: Gaining successful amplifications of seven VNTR and one SNP loci on all isolates, revealed different allelic formation. With the exception of SE5 with four alleles and SENTR7 with two alleles all other five VNTR loci didn't show heterogeneity. Nei's diversity index was calculated as 0.15 for SENTR7 and 0.58 for SE5,that made the final 0.66 simpson's diversity index. . While Salent 18, which initially was designed as a domestic VNTR locus containing a repeat unit of 18bps with no "tandem repeat" related heterogeneity manifestation among our collection, unexpectedly showed a well diverse (A or G) SNP marker. To our knowledge Adding Salent18 SNP findings in this study with 0.49 Nei's index(previously reported zero based on tandem repeat acquired data) improved our final Simpson's index up to the significant rate of 0.82 and increased our 6 distinctive phylogenetic cluster to 8 groups.

Conclusion: Our findings infers that using combinational methods with SNPs as molecular markers will provide a higher discriminative power, which consequently make a deeper insight into genotyping studies as well as phylogenetic relationships, provided that more genomic markers of SNPs incorporated in this method. SNP typing scheme could be a valuable complement to other gold-standard typing methods like PFGE and VNTR, that can be suggested as a fertile ground for future genomic studies of differences in the prevalence and virulence of different lineages.

Keywords: Salmonell Enteritidis, VNTR, SNPs, Molecular Genotyping

P768: **Use of Fuzzy Logic in Nanobiosensors for Masuring of IgG and IgM Proportion to Early Recognition of Veterinary Diseases**

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Background and Aim: Nanobiosensors can be used for detection and identification of different molecular proportion in nano scale. Discuss about use of nanotechnology in medicinal and its broad application in detection was introduced three decades ago. Few people would have thought that one day could be the key to solving medical problems found in the ninth digit after decimal.

Methods: Fuzzy Logic with extensive insight into the nature of statements and parameters involved in the deals and when debate on an issue instead of two 1 and 0, depending on the interval]0, 1] looks on. In this discussion, the triangular fuzzy numbers and fuzzy graph is used to check the levels of these antibodies are associated with health chart.

Results: Use of Fuzzy logic and fuzzy processors is important because of it can be obtained as a result of scientific research much precise. It has special effect in the early detection of disease through measurement values of IgG and IgM levels in the blood of stricken that is very important in safety food and veterinary medicine and it suggest, theory medical cost savings.

Conclusion: The use of fuzzy logic and fuzzy processors will be able to improve the power and accuracy of biological processors and it can be used to early detection of diseases. It is will be significant in prepare of food safety and livestock product .

Keywords: Nanobiosensors, Fuzzy Logic, IgG,IgM,Early Recognition

P769: **Identification of tetracycline-resistance genes in isolated Chlamydophila abortus in Chaharmahal va Bakhtiari province**

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Background and Aim: Ovine enzootic abortion, caused by C. abortus, is recognized as a major cause of lamb loss in sheep throughout Europe and is encountered in most sheep-rearing areas of the world. The biggest threat of human infection is pregnant women, because of the ability of C. abortus to colonize the human placenta. Tetracycline is generally used for the treatment of animal chlamydiosis. This kind of treatment must be used only to prevent a high rate of abortion at the time of the first outbreak in the flock, and then the animals must be vaccinated. In recently reviewed literature the discovery of the tet (C) islands represents the first identification of antibiotic resistance acquired through horizontal gene transfer in any obligate intracellular bacteria. Tetracycline-resistant Chlamydia suis have been isolated from pigs in several countries In vivo, such events are of particular public health concern if the tet (C) resistance gene is transferred from porcine chlamydial strains to human pathogens. As the tetracycline- resistant C.abortus did not reported yet, the main purpose of this study was to find it in sheep samples.

Methods: Samples were included 47 aspirated abomasums liquids of aborted fetuses. Extracted DNA were tested by Nested PCR and PCR to find 16s rRNA and tet genes respectively. Presence of C.abortus and tet (c) gene was confirmed by finding sequences homology using BLASTn.

Results: Twenty three samples out of 47 (51%) were positive as C.abortus. This result suggests that C.abortus is a main agent of enzootic abortion of ewes (EAE) in this area. Five samples among 23 samples were positive for tet (c) gene. High percent of homology (more than 98%) were shown in nucleotide sequences with previously reported sequence of tet (c) gene of extracellular bacteria in GenBank.

Conclusion: These results showed that C.abortus was a main agent of enzootic abortion of ewes and also confirmed the presence of tet (c) gene in C.abortus at the first time. Tetracycline is common used drug for the prevention and treatment of animal bacterial diseases in Iran. So if this resistance goes to expand, there's a concern for failing of our drug administration strategy in future. Tetracycline-resistant Chlamydia suis have been isolated previously from pigs in several countries. Such events are of particular public health concern if the tet (C) resistance gene is transferred from chlamydial strains to human pathogens. But more research should be planned and done to find whether tet (C) resistance gene is transferred in Chlamydiaceae family.

Keywords: tetracycline-resistance - Chlamydophila abortus - tet (c) gene

P770: **Detection of Brucella in human and animal serum samples by PCR and DNA extraction with boiling method optimization**

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Background and Aim: In most studies the molecular detection of Brucella genus used primers B4-B5 that amplify part of the 31-kDa Brucella abortus antigenic protein gene and can detect all species of Brucella. Due to the low levels of bacteria and presence of inhibitors such as antibodies and proteins in serum, detection of Brucella in serum by PCR samples, is often difficult. Therefore division of a low-cost approach with accurate results in molecular diagnostics for Brucella is of great importance. The aim of this study was to increase the sensitivity and specificity of primers B4-B5 and optimize the boiling methods in DNA extraction from serum for the diagnosis of brucellosis.

Methods: In this study, 38 human and 30 animals' sera were examined. All the samples were seropositive, and all were taken in the acute stage of the brucellosis disease. The specimens were tested by Wright, 2ME and Coombs Wright Test (in human samples) and antibody titer of each was determined. Their DNA was extract by the boiling method. By using BLAST software, variations on primers were performed to optimize them. Dilution of the extracted DNA was performed to reduce the effects of PCR inhibitors. The best dilution for PCR was determined and PCR was performed on all samples. For positive control, DNA extracted of the Brucella bacterium and for negative control, distilled water instead of DNA was used in the experiments.

Results: Results: This test was able to detect Brucella in serum in Wright's antibody titers 1/40 and higher, 2ME titers 1/40 or higher and Coombs Wright titers 1/20 and higher (human samples). From 68 positive sera, 54 samples were positive by PCR and the sensitivity of this test was 79.4%. Best dilutions of DNA extracted from the serum 1/200 were determined.

Conclusion: Modified primers B4-B5 have a sensitivity and specificity higher than the same primers in other reports. The modified primer can be used in future study to detect Brucella genus in clinical samples and as well as serum samples.

Keywords: Keywords: Brucella, PCR, B4-B5 primers, Boiling, Serum.

P771: **Colonization and occurrence, from blaZ gene of Staphylococcus aureus in the nasal cavity of healthy cattle, sheep and goats in Iran**

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Background and Aim: Staphylococcus aureus is a significant pathogen that can colonize the nares of different animals, and causing a broad range of infections in the various host. In Iran, neither the S. aureus nasal carriage rate nor the present genotypes or their antimicrobial susceptibly patterns are known in ruminants. The aim of the present study was performed to assess the proportion colonize and prevalence level of blaZ gene of S. aureus strains in the nasal cavity of ruminants .

Methods: In the present study, 79 healthy cattle, 78 healthy sheep and 44 healthy goats were screened for nasal S. aureus carriage and presence of blaZ gene by polymerase chain reaction (PCR).

Results: Among the 201 nasal samples obtained from ruminants tested in this study, the amount of S. aureus-positive nasal swabs from 79 cattle, 78 sheep and 44 goats was 4 isolate (5.06%), 11 isolate (14.1%) and 11 isolate (25%), respectively. Also, Among the 26 S. aureus isolates, 20 isolates (76.9%) harbored blaZ gene. The highest prevalence of blaZ was discovered among isolates from cattle, with 100% positive blaZ, in comparison to 81.8% in sheep(9 isolate) and 63.6% in goat (7 isolate).

Conclusion: As results, nares of healthy ruminants could be a reservoir of S. aureus isolates harboring penicillin resistance gene.

Keywords: Staphylococcus aureus; Nasal carriage; ruminants; Resistance gene

P772: **Complicated mastitis in a diabetic Dog- bacteriological and pathological findings**

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Background and Aim: Canine mastitis is an infrequent condition that occurs most commonly in the postpartum period due to ascending bacterial infection, but it may occur late in pregnancy and pseudopregnancy in diestrus cycle of the female dog. The main bacterial agents involved are Escherichia coli, Staphylococcus spp and Streptococcus spp. Ascending infection is the major cause of canine mastitis, however, traumatic lesions and hematogenous spread of bacteria from other sites of infection may also be involved in the pathogenesis of this disease. This clinical report, represent bacteriological and pathological aspects of a complicated mastitis due to ductitis obliterans in the right inguinal and left caudal abdominal mammary glands in an intact female diabetic dog.

Methods: A 4 year-old nulliparous mongrel dog was referred to Veterinary Hospital of Shahid Bahonar University of Kerman, Iran, with history of pseudopregnancy. Clinical examination showed erythma, tenderness and enlargement of right inguinal and left caudal abdominal mammary glands with bloody discharges.

Results: Blood sample was taken for hematological and biochemical evaluation. Complete blood count showed leukocytosis (25000/µl) with shifting to left. In biochemical analysis, increased glucose (190 mg/dl), cholesterol (210mg/dl), and triglyceride (190 mg/dl) level was detected and based to history the animal was suspected to diabetes mellitus. Urinalysis showed normal specific gravity and glycosuria was not seen. On the next step, fluid analysis with microscopic evaluation of the bloody discharge was done. Then exudate was submitted for bacteriological examination. Sample was cultured on blood agar, nutrient agar, MacConkey and BHI broth. Plates were incubated aerobically and anaerobically at 37°C with CO2 at a concentration of 5%-10% for 24h. The culture grew a coagulase-positive Staphylococcus isolate that was identified as S.intermedius. On the second check-up, fever, anorexia and depression was noted by owner and affected mammary glands were still hard, firm and painful. The second culture revealed Gram positive bacillus in blood agar and Gram negative bacillus in nutrient agar. The isolates were identified and confirmed by standard biochemical test as Bacillus cereues and Pseudomonas auroginosa. Histopathological study of mammary glands showed ductitis obliterans which is one of the diseases that cause mammary enlargement with cystic nature in intact female dogs and it can be considered when typical mastitis doesn't response to proper medical treatments.

Conclusion: Ductitis obliterans which is a characteristic of diabetic mastopathy in human medicine has been never reported in veterinary literature. Ductitis Obliterans or Mastitis Obliterans is a rare late manifestation of mammary ductal ectasia (MDE). MDE is an inflammatory breast disease of unknown etiology characterized by dilation of major mammary ducts associated with various degrees of inflammation and periductal fibrosis. In veterinary medicine, Pseudomonas aeruginosa is a common cause of chronic otitis externa in dogs, and treatment of these infections is becoming problematic because of the increasing number of multiresistant strains whereas the best therapeutic treatment was achieved by ceftazidime as we saw in present case. In conclusion it seems that clinical mastitis could be problematic in dogs if infection with pseudomonas auroginosa, diabetes and mammary ductal ectasia occurs contemporary.

Keywords: Pseudomonas aeruginosa, Staphylococcus intermedius, Dog, Mastitis

P773: **Detection of blaCTX-M, blaTEM and blaSHV genes in Escherichia coli isolates' obtained from buffalo feces in West Azerbaijan province**

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Background and Aim: Beta-lactamase enzymes are considered as the most important factor of antibiotic resistance among gram-negative bacteria. In recent years, the production of enzymes Extended Spectrum Beta Lactamases (ESBL) has been prevallent among bacteria and bacteria of animal origin is important in terms of public health. The aim of this study is to evaluate the presence of blaCTX-M, blaTEM and blaSHV genes in E.coli bacterial variants by using appropriate molecular techniques.

Methods: In this study, 105 isolates of E.coli, buffaloes' feces samples isolated from different parts of West Azerbaijan (33 isolates from Urmia, 33 isolates from Khoy, 24 isolates from Piranshahr and 15 isolates from Miandoab). In order to isolate E.coli variants, we used cultivation and biochemical characteristics as well as 23S rRNA gene-specific amplification methods. In the present study the presence of blaCTX-M, blaTEM and blaSHV genes were evaluated by using the PCR method.

Results: We observed blaCTX-M gene in 47 out of 105 isolates (44.7%) , blaTEM gene in 37 isolates (35.3%); while both blaTEM and blaCTX-M genes in 17 isolates (16.1%) of studied samples, respectively. blaSHV gene was not detected among the studied isolates.

Conclusion: The results of this study indicate that Buffalo gastrointestinal E.coli may be a source for ESBL, especially CTX-M and TEM type enzymes; so this should be considered in terms of public health and the transfer of resistance genes to pathogenic bacteria.

Keywords: E.coli, Extended Spectrum β Lactamases (ESBLs), blaCTX-M, blaTEM, blaSHV.

P774: **Pseudomonas aeruginosa infection in a young cat: A Case Report**

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Background and Aim: Pseudomonas aeruginosa is a Gram-negative, rod-shaped bacterium which causes a wide range of opportunistic infections. Cystitis, pneumonia, ulcerative keratitis and sometimes septic shock caused by P. aeruginosa, occurs in cats and dogs with variable mortality rates. It is also responsible for severe nosocominal infections, in specific respiratory infections in patients suffering chronic pneumonia, sepsis and meningitis. This organism causes a variety of infections in a wide range of animals and susceptibility to the infection depends on the host's immune, nutritional status, environmental conditions unfavorable for the host and genetic factors.

Methods: A seven-month-old female cat was admitted to small animal hospital of Shahid Bahonar University (Kerman, Iran) with urgent signs of dyspnea, respiratory distress and collapse, %10 dehydration and fever. Animal was on her sixth day of Ampicillin (200 mg IM q12h) and Dexamethasone (0.5 ml IM q24h) therapy for pulmonary infection with signs of anorexia, lethargy, cough, high fever (39.9°C) by a private clinician. In further examination, Cardiac arrhythmia, expiratory crackles and wheeze sounds were observed. Shock treatment including oxygen therapy (by Endotracheal tube), fluid therapy, bronchodilator, and broad-spectrum antibiotic was performed but the animal did not respond to our treatment and passed away due to severe sepsis.

Results: On postmortem examination, animal showed severe pulmonary disease and septic shock symptoms. Respiratory aspirates, were submitted to microbiological lab. Suspect materials were cultivated in Blood agar and MacConkey agar plates, and incubated aerobically at 37°C for 48 hours. Samples appeared pale and non-lactose fermenting colonies on MacConkey agar, and grey, round and non-haemolytic colonies on blood agar. Bacterium was Catalase-negative and Oxidase-positive in biochemical tests. Then a smear was taken and examined under the microscope; and gram negative medium- sized rods were observed. Afterward to confirm our diagnosis, the isolates were cultured on nutrient agar at 37°C and 42°C. Bacteria growed at 42°C with blue-green fluorescent colonies under UV light, diffused in the medium which confirmed our diagnosis of Pseudomonas aeruginosa.

Conclusion: Pseudomonas aeruginosa, could cause severe infections that can be difficult to deal with if not diagnosed early. These infections usually occur in patients with chronic diseases, characterized by impaired defense mechanisms, patients submitted to invasive therapeutic treatment or with immunodeficiency. The clinical feature of this case was found by respiratory symptoms. The presence of pneumonia is usually associated with a worse diagnosis, together with the presence of shock. Since Pseudomonas aeruginosa is highly resistant to antibiotics and disinfectants, found everywhere and grows easily, prompt antibiotic treatments including fluoroquinolones as second-line antibiotics should be administered. Also pet owners and their family members may be at risk from this pathogenic agent. Children, and other people with immunodeficiency disorders should avoid contact with pets with clinically suspected Pseudomonas aeruginosa.

Keywords: Pseudomonas aeruginosa, cat, pulmonary infection.

P775: **Pyothorax associated with Salmonella spp. in a cat: A Case Report**

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Background and Aim: Feline pyothorax or feline thoracic empyema is a life-threatening emergency describing infection of the pleural space characterized by accumulation of a purulent exudates. Bacterial isolates from the majority of cases of pyothorax are polymicrobial and similar in composition to the normal feline oropharyngeal flora. Isolates include obligate and facultative anaerobic bacteria; Bacteroidaceae, Fusobacterium spp., Peptostreptococcus spp., Clostridium spp., Actinomyces spp., Eubacterium spp., Propionibacterium spp., Filifactor villosus, Pasteurella multocida, Streptococcus spp. and Mycoplasma spp. Less than 20% of cases of feline pyothorax are caused by infectious agents other than oropharyngeal flora including Staphylococcus spp., Rhodococcus equi., Nocardia spp., enteric Gram-negative organisms (Escherichia coli., Salmonella spp., Klebsiella spp., Proteus spp.) and non-enteric Gram-negative organisms (Pseudomonas spp.) and protozoa (Toxoplasma gondii). However isolation of Salmonellae strains from feline pleural effusion is a very rare entity which is described in present clinical report.

Methods: A two-year-old female cat was admitted to small animal hospital with history of anorexia, weight loss and chronic diarrhea from last month and a recent history of severe dyspnea. In clinical examination, pale mucous membranes, severe anemia and muffled thoracic sounds were noted. Radiography showed severe plural effusion. Thoracocentesis was done and drained straw colored gelatinous fluid submitted for bacteriological and cytological examinations.

Results: On cytology, the effusion was exudate and proliferation of mononuclear cells especially lymphocytes and reactive monocytes containing numerous coccobacillus bacteria were noted. The exudate was cultured on suitable medium for bacterial isolation. The isolates were identified and confirmed by standard biochemical test as Salmonella spp. The animal was died before thoracostomy tube was inserted for antibiotic therapy. On postmortem examination, pyothorax with extensive deposition of fibrin on pleural membranes and tracheobronchial tree and pulmonary congestion were seen. No significant abnormality was found in other organs. Based to animal medical history, it seems that hematogenous route was the main route for spreading of salmonella spp from gastrointestinal tract to thoracic cavity in this case.

Conclusion: Feline salmonellosis could occur in different clinical forms including gastroenteritis, bacteremia/endotoxemia, pyrexic episodes accompanied by vomiting, localized infection in extraintestinal organs such as conjunctivitis, abortion, stillbirths, and fading kittens. Approximately one percent of empyema and pyothorax cases, reported to cause by Salmonella spp in immunocompromised people, however Pyothorax associated with Salmonella spp infection is a very rare entity in feline medicine which is described in this case report.

Keywords: Pyothorax, Salmonella spp, cat

P776: **Prevalence of staphylococcal mastitis and their antibiotic susceptibility in dairy farms around Khoy**

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Background and Aim: Mastitis, inflammation of the parenchyma of mammary gland, regardless of the cause, is characterized by physical, chemical and bacterial changes in the milk and pathogenic changes in the glandular tissue. Reduction in milk production from affected quarters and the consequence for economical loss, makes it serious disease with which the dairy industry used to encountered. Staphylococcus aureus is the most common bacterial agents of mastitis in dairy cows that is isolated from contagious mastitis. The main purpose of this study, was the evaluation of the outbreak of mastitis caused by Staphylococcus aureus and its antibiotic susceptibility pattern in dairy farms in Khoy.

Methods: The laboratory techniques used in this research consisted of: sampling of milk under sterile condition, differential tests for the positive samples routin culture and isolation methods and antibiotic sensitivity test.

Results: In this research, 107 heads of dairy cows showing clinical mastitis were selected. Of these, 21 cases (19.62%) were identified with mastitis caused by Staphylococci, consisting of 12 cases (11.22%) of positive coagulase Staphylococci and 9 cases (8.4%) negative coagulase Staphylococci. The relationship between prevalence of mastitis and season of contamination was meaningless ($p>0.05$) but it was meaningful during breeding period($p<0.05$). Antibiotic sensitivity test determined the most sensitivity rate to Enrofloxacin, Fleurophenicol and Lincospectin and the least sensitivity rate to Penicillin G, Canamycin, Streptomycin and Tylosin.

Conclusion: It was concluded that microbiological and antibiogram studies are necessary for treatment and control of bacterial mastitis.

Keywords: clinical mastitis, prevalence, Staphylococcus, antibiotic sensitivity, khoy

P777: **Comparative effect of antibiotics on Escherichia coli isolated from broiler farms contaminated with colibacilosis in Khoy city by culturing method and antibiogram**

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Background and Aim: Colibacilosis overall economic condition of diseases in the broiler chicken breeding units of bacterial resistance due to indiscriminate use of drugs years ago this disease is most common in farms. The cause of this disease associated with Mycoplasma infection (Mg), are called as the CRD Complex or chronic respiratory disease. Against Mycoplasma Galysepticum that is spread through eggs, Colibacilosis is not the real overall an Egg born. Disease at early age is particularly high occurrence of the first 3 weeks. Purulent pus into the air sacs and visceral organs is clinical and autopsy signs of the disease. Given the economic importance of this disease in order to achieve effective therapeutic strategies, antibiogram testing seems necessary. The purpose of this study, determine of antibiotic susceptibility of broiler flocks with Colibacilosis overall mood in the Khoy city.

Methods: In this study, 233 samples were examined clinically suspected Colibacilosis overall in total 192 positive samples (82/41%) and 41 negative samples (17/59%) and It was transferred to plates containing medium. Medium used in this way, Bloodagar, Mac conkey agar, urea agar, SIM, gelatin and the MR-VP. Then the colonies in agar plates were transferred to the medium of Muller Hinton Agar that total of 9 antibiotic disk diffusion method was used.

Results: Antibiogram results of samples that were most susceptible to Enrofloxasin (Bytryl) and Lincomycin 72/10% and 68/24%, respectively and the least sensitive to the Oxy-tetracycline (99/57%), Tylozin (94 / 42%) and neomycin (93/99%) belonged.

Conclusion: It seems that proper implementation of quarantine operations and increase the biosecurity and operation of antibiogram, are important steps in reducing and controlling the Colibacilosis disease.

Keywords: Colibacilosis, broiler chicken, antibiogram, Khoy

P778: **Seroprevalence of bluetongue virus infection among sheep in Khoy, Iran by Competitive ELISA**

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Background and Aim: Bluetongue is one of the viral diseases in ruminants. The type species of the genus Orbivirus within the family: Reoviridae. It is transmitted by colicoides.

Methods: This study was conducted on 200 sheep's blood samples from 19 sheep flocks of 7 villages in Khoy city located in North-Western of Iran. 160 out of 200 sheep were ewe and 40 were male. The objective was describing the prevalence and distribution of serum antibodies to Bluetongue virus (BTV) in a sample. Competitive ELISA was applied to detect antibodies.

Results: 134 samples (67%) were positive and 66 samples (33%) were negative. 23 of males and 111 of females were positive (57.50% and 69.37%, respectively). The difference prevalence of antibodies in serum between male and females was not significant($p>0.05$). The relationship among prevalence antibodies in serum and age-groups was significant (phi= 0.59) and ($p<0.05$).

Conclusion: From this study it is concluded that the bluetongue antibodies presence in the sheep sera from Khoy sheep flocks and can cause a disease.

Keywords: Seroprevalence, bluetongue virus, sheep, competitive ELISA,Khoy

P779: **Genotyping Escherichia coli producing Extended-spectrum Beta-Lactamase (ESBL) isolates in human and animal origin**

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Background and Aim: Escherichia coli (E. coli) species are able to produce extended-spectrum β-lactamases (ESBLs) that cause high resistance to the beta-lactam antibiotics. Therefore, determining the antibiotic susceptibility patterns in resistant organisms is necessary for suitable therapeutic approaches. In contrast, this study evaluated the genotype variation of E. coli produced extended spectrum beta-lactamase (ESBL) isolated from cases of diarrhea, control separated of calves and urinary tract infection (UTI) human using Entro bacterial repetitive consensus (ERIC).

Methods: Production of ESBLs in our study among E. coli isolates was determined by the combination disk technique using antibiotic disks containing Ceftazidim (30µg), cefotaxime (30µg) and cefpodoxim (30µg) either alone or in combination with Clavolanic acid (10µg). Then genetic background determined in microorganisms produced ESBLs and evaluated clonal relation in those using ERIC-PCR.

Results: After determination of zone of inhibition for the Antibiotics discs to that of the discs plus Clavolanic acid combined discs, 10 ESBL producers were identified. 7 separated were from species of urinary tract infection (UTI) and 3 separated from healthy and disease calves.

Conclusion: The ERIC polymorphism patterns obtained as illustrated in a dendrogram showed a significant discriminatory finger print among the 10 E.coli strains. Nearly every isolates had a unique fingerprint.

Keywords: E.coli- ESBL- ERIC- HUMAN- CALVES

P780: **comparison the prevalence of iut A gene in Escherichia coli isolated from avian colibacillosis and human urinary tract infection**

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Background and Aim: Colibacillosis is one of the most common diseases in birds raised for meat and eggs. Recently some researches report that there is a link between APEC (Avian Pathogenic Escherichia coli) and human diseases that are caused by Escherichia coli strains like urinary tract infections(UTIs). Common similar diseases patterns and phylogenetic background indicate a common presence of some virulence associated genes among APEC and UPEC (Urine Pathogenic Escherichia coli) strains and a genetic relationship between them. Extra pathogenic Escherichia coli (ExPEC) strains like APEC and UPEC may possess multiple iron acquisition mechanisms. more studies have shown the pathogenesity of APEC has been correlated with the aerobactin iron uptake system. The aim of this study was investigation and comparison the presence of iut A as a aerobactin siderophore receptor gene in APEC and UPEC strains.

Methods: in this study 246 Escherichia coli isolated from avian colibacillosis and 20 E.coli islated from UTIs were evaluated. To confirm strains, standard biochemical tests were used. The pure colonies were transferred to nitrocellulose membrane by a replica blotting method and colony hybridization assay carried out by using 32P labeled iut A gene as probe. This fragment was labeled by the random priming method by using multiprime DNA labeling system and Statistical analysis was performed using $\chi 2$ test.

Results: the results of this study showed 72.8 % of APEC and 75% of UPEC isolates harbored iut A gene. According to results of this study the prevalence of iut A gene is very high in both APEC and UPEC strains.

Conclusion: although a few UPEC strains evaluated in this study, and statistical analysis is difficult, since the both strains may have similar challenge to establish infection in extra intestinal locations, probably they share a similar content of virulence genes. Thus APEC strains can serve as a source of human ExPEC or as a reservoir of virulence genes for human ExPEC.

Keywords: tra pathogenic E.coli, virulence associated gene, iut A

P781: **Virulence-associated genes in Escherichia coli isolates from poultry colibacillosis**

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Background and Aim: Although Escherichia coli are comensal bacteria of intestine of human and animals, some specific strains possess certain virulent factors are able to cause diseases in human, animals and birds. Colibacillosis in all its forms is one of the most significant infectious diseases in production birds, resulting in large annual losses for the poultry industry. exploitation of common APEC (Avian Pathogenic Escherichia coli) markers as the target of vaccines or for diagnostic tools can yield measure with general applicability to control of colibacillosis

Methods: Two hundred thirty four Escherichia coli isolated from avian colibacillosis (APEC) and 54 fecal E. coli isolates from the feces of apparently healthy birds (AFEC) were used in this study. To confirm the E. coli strains, standard biochemical tests were used. presense of iut A, iss, hly F, omp T and iro N genese were evaluated by a set of pentaplex PCR. Statistical analysis was performed using χ 2test. Significance was accepted when the P-value was ≤ 0.05

Results: Out of 234 E.coli strains in pathogen group 157 (67.4%) isolates were positive for iut A, 180 (77.3%) for hly F, 159 (68.2%) for iss, 152 (65.2%) for iro N and 170 (73%) for omp T, while, out of 54 fecal isolates, 32 (60.4%) isolates were positive for iut A, 20 (37.7%) for hly F, 13 (24.5) for iss, 12 (22.6%) for iro N and 20 (37.7%) for omp T

Conclusion: Indeed of more studies on Escherichia coli isolated from poultry, there are no certain traits to define APEC pathotype. Plasmid mediated horizontal gene transfer, cause to a large extensive diversity within APEC strains. In this study significant differences were shown in the prevalence of iss, hly F, omp T, iro N in APEC and AFEC strains.So these genes can be used as a marker to identify traits that predict avian pathogenic E. coli. However presence of DNA sequences associated with virulence factor does not mean that, it is expressed and pathogenesis of these isolates after inoculation to respiratory tract of chick should be evaluated.

Keywords: Avian pathogenic Escherichia coli, multiplex PCR, virulent factors

P782: **serodiagnosis of brucellosis among high risk people in Shahrekord**

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Background and Aim: Brucellosis is one of the most important and worldwide spread zoonosis. In humans, brucellosis is a debilitating and chronic disease, which may affect a variety of organs. Brucella spp. are bacteria that affect particularly individuals consuming unpasteurized dairy products, abattoir workers, veterinarians, farmers and the disease is easily acquired by people involved mainly in laboratory routine. Laboratory testing is therefore very important for a correct identification of the disease in humans. Goals: The purpose of this study was to investigate and serodiagnosis of brucellosis among high risk group individuals, consisting of slaughterhouse workers, farm workers, laboratory workers and veterinary students.

Methods:: A total of 130 serum samples were collected from different individuals (slaughterhouse workers (n= 40), farm workers (n= 30), laboratory workers (n= 20) and Veterinary students (n= 40)) and Rose Bengal Test was performed on all samples, in positive cases with Rose Bengal, Wright test, to determine antibody level, was carried out.

Results: Rose Bengal Test was negative among slaughterhouse workers, laboratory workers and veterinary students but out of 30 serum samples from farm workers, 15 samples were positive by the Rose Bengal Test. Wright test was performed on this 15 samples and in ten samples, Wright's titer > 1/80 and in five samples Wright's titer < 1/80.

Conclusion: In this study, the disease was higher among farm workers as compared to other occupational groups and this indicated the importance of direct contact with infected animals or with aborted fetuses, placental membranes or fluids, and other vaginal discharges, in transmission of brucellosis from animal to human.

Keywords: serodiagnosis - brucellosis - high risk people

P783: **The Role of Biofilms in Veterinary Medicine and animal disease**

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Background and Aim: Recent studies done by electron microscopy and molecular biology techniques have allowed to explain in detail aspects of microbial biology to establish that the majority of bacteria remain attached to surfaces within a structured ecosystem known as biofilm. This is defined as a community of microorganisms that grow inside a matrix of exopolysacharides attached to an inert surface or living tissue, and may be comprised of a single bacterial species or a range of different species, including fungi. Biofilms play an increasing role within the medical and veterinary community.

Methods: Due to the increased resistance of a biofilm, they can have direct and indirect effects upon a range of infections and diseases including chronic non-healing wounds, implant/prosthesis infection and mastitis. These problems can have significant effects on other industries, for example mastitis can have a detrimental effect on milk yield in the dairy industry.

Results: There are many evidences that relate biofilm to different infectious processes, although the mechanisms that produce disease are not yet fully understood. It has also been observed the formation of these structures in the affected tissues and from which groups of bacteria are released into the bloodstream where they can evade the phagocytic cells of the immune system, besides being resistant to treatment with antibiotics. These issues are addressed in this review focused on bacteria of veterinary importance, in order to understand their participation in diseases such as pneumonia, myocarditis, epididymitis, mastitis, abortion, otitis, conjunctivitis, polyarthritis and septicemia in domestic animals.

Conclusion: The degree of severity biofilms can cause increases the pressure on the veterinary industry to diagnose and treat infections and diseases quicker and with more effective results. With maturity, biofilms may become more resistant to the effects of antimicrobials which make the infection harder to treat. As elaborated on in previous chapters, many antibiotic therapy treatments currently used to treat bacterial infections are aimed at planktonic bacterial cells as opposed to cells encased in a biofilm; this makes their treatment increasingly problematic. Without adequate diagnostic and treatment protocols to treat veterinary biofilms, their impact will remain a significant challenge.

Keywords: Biofilm, Bacteria, animal disease, Veterinary Medicine

P784: **Cloning of VP1 gene of FMD virus type O/IRN/2010 in Pet28 vector**

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Background and Aim: The Foot and Mouth Disease Virus (FMDV) cause a lot of economical loss in domestic animals. Foot-and-mouth disease (FMD) is a highly contagious disease of livestock and according of the OIE guideline the most important infectious agent for cloven-hoofed animals. PET vector is one of the best systems for cloning and expression of proteins in E.Coli. The aim of this study is isolation of FMDV type O/IRN/2010 VP1 gene by RT-PCR and its ligation into the PET28 prokaryotic vector in order to express the VP1 protein as a peptide vaccine.

Methods: The FMDV type O/IRN/2010 isolate was collected from cattle in Qom and cultured on pig kidney cells (IBRS-2) in the Razi Vaccine and Serum Research Institute of Iran. The FMDV was propagated in BHK21 cell line and the genomic RNA was extracted by RNeasy Mini Kit (Qiagen) and used as template for RT-PCR in order to amplification. The primer was designed for amplification and cloning of the VP1 gene based on the published FMDV O/2010/IRN sequence (Accession number:). There are BamH I and Xho1 sequences and three overhanging nucleotides at the start of forward and reverse primers, respectively. The forward primer contains the kozak consensus sequence and start codon. The reverse primer contains two stop codons. The extracted RNA was reverse transcribed and amplified using the VP1 gene, the specific primer pair and the AccuPower one-step RT-PCR kit (Bioneer). The purified VP1 gene was sub-cloned into the unique BamH I and Xho1 cloning sites of the PTZ57R/T vector (Fermentase) to construct the VP1 gene cassette and named PTZ57R/T-VP1. The DH5α strain of E.Coli was transformed with the vector using heat shock and the CaCl2 method. Positive clones were confirmed by restriction enzyme digestion. The digested FMDV O/IRN/2010 - VP1 gene from PTZ57R/T vector was used for ligation in digested Pet28 and confirmed using double digestion of VP1 gene. Then the Pet28- VP1 gene construction was transformed in BL21 strain of E. Coli.

Results: RNA genomic of the FMDV type O/IRN/2010 was amplified using the specific primer pair for VP1 gene and the digested VP1 gene was sub-cloned in T/A vector (PTZ57R/T). Then the digested VP1 gene from T/A vector was sub- cloned in Pet28 vector and confirmed by double digestion using BamH I and Xho1restriction enzyme.

Conclusion: The Pet28-VP1 gene construction can used to express VP1 protein in order to peptide vaccine in the future research.

Keywords: FMD Virus, gene, Cloning, Pet28, Vector

P785: **Sequence and phylogenetic characterization of the fusion genes of the Newcastle viruses isolated in Iran during 2009-2011**

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Background and Aim: Despite routine vaccination programs against Newcastle disease (ND), sporadic cases have occasionally occurred and remain a constant threat to commercial poultry. So the aim of this study was designed to characterize the phylogenetic analysis and sequence of 10 NDV isolates which were obtained and collected from infected broiler chicken farms in Fars province, Iran during 2009-2011.

Methods: At this point, The NDV isolates were approved at the department of virology, Razi vaccine and serum research Institute Shiraz, Iran, and the fusion (F) gene sequences of ten NDV strains isolated from outbreaks on poultry farms were determined. The viruses were confirmed as NDV by hemagglutination inhibition assay. The isolates based on the sequence and phylogenetic analyses of partial F gene were genotypically analyzed (RT PCR). In the present investigation, the pathogenicity of NDV strains was determined by internationally recognized test Mean Death Time (MDT). The subsequent phylogenetic analysis was implemented using MEGA and the phylogenetic tree.

Results: Analysis based on F gene showed that characterized isolates possess three different types of protease cleavage site motifs and appear to show maximum identities with isolates in the region. The results of RT-PCR and MDT showed that 10 samples were positive for NDV, (60% velogenic, 30% mesogenic and 10% lentogenic).

Conclusion: The results of the phylogenetic analysis showed that 10 NDV isolates from Iran belong to the class II, genotype III viruses. A better understanding on the genotypical history of NDV might be able to facilitate the development of more effective vaccines.

Keywords: Newcastle disease virus, Fusion protein, Phylogenetic analysis, Mean death time.

P786: **molecular genetic differentiation by PCR-RFLP and antimicrobial susceptibility testing of avian Escherichia coli in khozestan province**

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Background and Aim: The purpose of this study was to identify serovars of Escherichia coli using seotyping , PCR-RFLP and also to determine their antimicrobial susceptibility in commercial broilers in Khuzestan province.

Methods: A total 50 isolates of E. coli were recovered from Commercial broiler flocks, from April 2010 till July 2010 in khozestan province. Isolation and identification of E. coli were performed by standard bacteriological methods. Total isolates were subjected for serotyping by using polyvalent and monovalent antisera. Twenty antibiotics were used in this study. Antimicrobial susceptibility determination was routinely tested by the single-disc diffusion method. Genomic DNA of these serovars was extracted by the standard phenol-chloroform method. The entire coding sequence of fliC gene and PCR was standardized. PCR products were detected by electrophoresis. The amplified fliC gene was cleaved with HhaI restriction endonuclease. To further characterize isolates we developed RsaI restriction endonuclease .

Results: Serotyping of isolated E. coli includes: O1 (34%), O2 (20%) , O6 (20%) ,O128 (4%),O8 (2%), None typable(20%). The highest rate of resistance was against Nalidixic acid , Erythromycin, lincomycin (100%) ,Amoxicillin (96.6%), Flumequine (91.6%), high sensitivity to ceftriaxon (100%), ceftazidime (96.6%) were noticed. Most E. coli strains tested produced a single band between approximately 1.1 and 2.2 kb that is variable in O - serogroups.a total of 14 restriction patterns were observed after RsaI restriction and 13 restriction patterns were obtained for HhaI. In Comparison of two enzyme RsaI and HhaI , we demonstrated that discriminating of RsaI is better than HhaI to differentiation of E.coli isolated.The same Restriction Pattern had obtained for Standard serotypes O128 and O1 with HhaI and These strains are discriminating with RsaI and each of three isolate relieved difference Restriction pattern.

Conclusion: frequently of O serogroups of E.coli are different in poultry. Results showed that O1,O2,O6 serogroups were predominant. The significant increases in the incidence of resistant isolates were observed which indicated that probably due to increased use of antibiotics in poultry.Finally our study comparing discriminatory power of the assayes showed that PCR-RFLP was useful, a feasible and rapid method for identification and subtyping and tracing E.coli serovars. Since the whole process for sera preparation and serotyping itself is expensive, laborious and time-consuming. However, it should be noted that the great value of traditional serotyping is important for the epidemiological study of a high variety of Escherichia coli serovars.

Keywords: Escherichia coli, serotyping, fliC gene,PCR-RFLP, antimicrobial susceptibility, khozestan province

P787: **Isolation of an antibiotic resistant fecal streptococcus from Ostrichs**

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Background and Aim: Ostrich breeding is increasing because of its novelty in Iran, a good property of its meat and some miscellaneous usage of its fats, Gelatins ,bones and feathers ,Ostriches are held on the lands in groups population 3-2 female against 1 rooster, they fed with some nuts and herbs but they would be caprophagic and have a bit to the feces and other things in abnormal or stressful environment. In current study an antibiotic resistant fecal streptococcal infection outbreaks' were reported in the ostrich.

Methods: The affected birds show some clinical signs of depression, loss of body weight, lameness, and head tremors , upper respiratory film and sever inflammation and hyperemia, and poor responses to the widespread antibiotics, In autopsy finding of 3 death birds the sever necrotic plaque were seen along the trachea and pharyngeal tonsils and livers also the hearts were smooth with sever petechial hemorrhages, regarding to the prior cases some samples from the trachea and whole blood prepared for bacterial exams.

Results: The blood impression smears provide a presumptive diagnosis of streptococcosis and Fermentation of mannitol, sorbitol, arabinose, and growth on MacConkey agar with the history results for a fecal streptococcus(Enterococcus spp) infection. In antibiogram test for isolated streptococci the result showed antibiotic resistance response for lincopec, gentamox ,erythromycin, oxytetracycline, chlortetracycline,tetracycline.

Conclusion: The fecal streptococcus(Enterococcus spp) infectionin avian species is worldwide distributed, Enterococci are ubiquitous in nature and commonly found in various poultry environments. Enterococcus spp. are considered normal micro flora of the intestinal tract of poultry .A low percentage (16.67%) of poultry meat contamination with E. faecalis has been found in ready-tocook products; However, later studies found a much higher percentage of gram-positive cocci including Enterococcus spp. present on meat samples at processing so except the mortality it can cause some antibiotic resistancy.

Keywords: Ostrich ,Fecal streptococcus ,Antibiotic ,Resistant

P788: **comparision Study of presence eight correlated gene with avian pathogenic Escherichia coli in human uropathogenic Escherichia coli**

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Background and Aim:: A wide range of extra intestinal infections in humans and vertebrate animals are created by the Extra intestinal E.coli strains (EXPEC). Including the APEC (Avian pathogenic Escherchia coli) and urinary tract infection (Uropathogenic Escherchia Coli) noted in humans. Recent studies indicate that E.coli of the animal is the suitable source for emergency presence of UPEC.the aim of study is evaluation of the presence virulence factors between APEC in UPEC.

Methods: This study investigated the presence of associated genes (astA, iss, irp2, papC, iucD, tsh, vat, cva / cvi) with APEC in UPEC of isolated from patients with UTI west of Tehran by using multiplex PCR. The correlation was evaluated by chi-square test.

Results: All isolates of urinary tract infection were having with different genes astA (13 of 100), iss (11 of 100), irp2 (33 of 100), papC (3 100). None of these genes isolated iucD, tsh, vat, cva / cvi carrying did not. While the 28 isolates of APEC study of genes were required astA (24 of 28), iss (27 of 28), irp2 (15 of 28), papC (23 of 28), iucD (24 of 28), tsh (24 of 28), vat (24 of 28), cva / cvi (14 of 28).

Conclusion: The results showed that there are meaningful relation sheep between the presence of genes for virulence astA, iss, irp2, papC, in APEC and UPEC. genes iss, having the highest frequency in both strains as well as the frequency of 33% gene irp2 in strains of UPEC are proposed likely the most important factors that contributing in the presence of extra intestinal Escherichia coli live.

Keywords: Uropathogenic Escherichia Coli, avian pathogenic Escherichia coli, virulence factors, Multiplex PCR.

P789: **Molecular and phylogenic analysis of gene encoding protein F of Newcastle virus isolated from migratory birds Bushehr**

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Background and Aim: The Newcastle disease virus(NDV) is a member of the Paramyxoviridae family,genus paramyxovirus.Despite the virus is spread by migratory birds throughout the world and it considered cause of the disease in poultry industry ,despite the survay of Pathotyping viruses isolated from migratory birds to prevent transmission is necessary

Methods: Samples of present research collected from wetlands of Boushehr province in south of Iran in 2009-2010.Virus isolation and characterization of the samples were performed at the Razi Vaccine and Serum Research Institute,Shiraz,Iran.Pathogenic viruses was diagnosed by MDT and RT-PCR methods.In MDT method,dilutions of 10-4 to 10-9 from the virus in embryonated eggs were injected and seven days of being alive or dead embryos were studied.RT-PCR was performed whit the use of 535 bp motif of primer that contain Cleavage site of F protein.then the Amino acid sequencess were used to draw the phylogenic tree

Results: MDT method showed that all of 10 samples were detected as lentogenic strain with low virulent and so,in RT-PCR method all of the samples in the special motif were positive.After analysing the phylogenic tree and comparing the results with the registered strains in gene bank,it was shown that all the samples were similar to the lentogenic ones

Conclusion: Birds migrate from the Northern Hemisphere to the Southern,as a key factor in transmission of Newcastle virus in poultry industry. In this survay Lentogen viruses isolated from migratory birds that it transfer the disease to poultry industry,which need for prevention in poultry

Keywords: Newcastle Viruses,Paramyxovirus,MDT,RT-PCR

P790: **ANTIMICROBIAL RESISTANCE OF PROTEUS ISOLATED FROM POULTRY INTESTIN IN ILAM**

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Background and Aim: Poultry intestine could be a source for pathogenic and non pathogenic bacteria. Proteus is one of the most frequent isolate bacteria form animal intestine. This bacterium is the second important bacteria in urinary infection in human. The emergence of antimicrobial-resistant proteus is associated with the use of antibiotics in animals raised for foods, particularly those of animal origin. Resistant bacteria can be transmitted to human. This study was undertaken to estimate the antimicrobial resistance of proteus isolated from chicken intestine before they slaughtered.

Methods: From February 2011 to September 2012 a total of 370 chicken intestines were sampled from 74 randomly selected flocks with cotton sterile swab and examined for the presence of Gram negative bacteria (Salmonella, Proteus, Ecoli). 54 Proteus isolates were tested for their susceptibility to 13 selected antimicrobial agents' (Ciprofloxacin, Doxycycline, Cefotaxime, Oxytetracycline, Norfloxacin, Kanamycin, Nalidixic acid, Amikacin, Gentamycin, Imipenem, Piperacillin, Ampicillin, Ceftriaxone) by the disk diffusion test.

Results: All of the isolates were sensitive to Gentamicin. Fifty two (96%) isolates were resistant to two or more antibiotics that we used in this study. Resistance to Nalidixic acid (93%), Doxycycline (91%) and oxy tetracycline (89%) was the most frequent.

Conclusion: The high level of antibiotic resistance of proteus isolates in this research is an indication of Unrestrained and continuous use of antibiotics in animals. In the other hand the results showed the possible significance of chicken as a source of multiple antimicrobial-resistant proteus and this bacteria can be a worldwide problem both for veterinary and public health sectors.

Keywords: proteus, poultry, antimicrobial-resistant

P791: **Effect of Savoury (Satureja hortensis) essential oil on bacterial load of poultry feed**

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Background and Aim: Bacterial microorganisms grow in poultry feed and there is possibility of microorganisms transferring into their body. Bacterial contamination of poultry feed may reduce quality and it is caused number of diseases. There are common methods for controlling microorganisms in the feed but they are not effective always and have caused side effects in poultry and humans. Extensive studies had been on the effects of anti-bicrobial and antioxidant herbs done including Satureja hortensis essential oil. Antibacterial and antifungal properties of Satureja hortensis essential oil of fennel had been proven.

Methods: In this study is investigated the effect of Satureja hortensis essential oil on microbial load in poultry feed. The Satureja hortensis essential oil obtained by distillation method and it was prepared different dilutions. The certain amounts of various dilutions were added to poultry feed. Bicrobial load was calculated to 2 weeks.

Results: Results were analyzed with using Minitab software. The Satureja hortensis essential oil in 250 µl dilution had maximum effect and bicrobial load to minimize during 24 and 48 hours from start adding essential oil to the feed. Bacterial colonies counting grown on plate show that from two hours Satureja sahendica reduce bacterial contamination. These effects persist since two weeks and increasing Satureja sahendica concentration was more reduction bacteria counting (P <0/05).

Conclusion: The Satureja hortensis essential oil may be suitable alternative for chemical materials in poultry industry. This combination is natural perfectly, so it could be so safe but a broader investigation of the possible side effects on the poultry is essential.

Keywords: poultry feed, Bacterial load, essential oil, Satureja hortensis

P792: **Isolation of Escherichia coli O157: H7 bacteriophage from agricultural soli**

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Background and Aim: Bacteriophage treatment offers a possible alternative to conventional antibiotic therapy against multidrug-resistant bacteria. Phage therapy has already been proven to be advantageous as these are very specific, accurate and potent than antibiotics. The aim of this study was isolation of E. coli O157: H7 specific phages.

Methods: To isolate of these phages, samples of soil, agricultural soil enrichment with animal manure and waste were collected. One-gram portions of each sample were inoculated into 5-ml exponential cultures of E. coli O157: H7 and the cultures were grown in Luria-Bertani (LB) medium supplemented with 5 mM MgSO4 (LBM) overnight at 37°C. After overnight growth, the cultures were vortexed, centrifuged at 10,000 × g for 10 min, and filtered (pore size, 0.45 μm) to remove cellular debris. Phage activity in the supernatant was tested by a spot assay that entailed placing 10 ul of the supernatant on LB agar seeded with E. coli O157: H7. The plates were checked for plaques after 18 h at 37°C. Lysis-positive supernatants were serially diluted, and plaques were isolated and purified using a soft agar overlay technique with E. coli. Presence of the E. coli O157: H7 phage was confirmed by Electron Microscope. Specificity of becteriophage isolated was investigated.

Results: In this study E. coli O157 phage was isolated from agricultural soil enrichment with animal manure. This phage produced clear plaques (0.2- to 0.3-mm-diameter) on E. coli O157 but not on the other bacteria. The number of phage was calculated 3 × 104 PFU/ml. Phage virions were shown and confirmed By TEM.

Conclusion: The virulent phage that we isolated in this study may be used for biocontrol of E. coli O157 in animals and fresh foods without compromising the viability of other normal flora or food quality

Keywords: Bacteriophage; E. coli O157: H7

P793: **Coagulase gene polymorphism detected by PCR-RFLP in Staphylococcus aureus isolated from healthy horses in Iran**

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Background and Aim: Staphylococcus aureus is a common opportunistic pathogen in many species, including humans and horses. Molecular epidemiological analysis such as Polymerase Chain Reaction-Restriction Fragment Length Polymorphism has been crucial in our understanding of the emergence and dissemination of microorganism and our evaluation of changes in clinical epidemiology. The aim of the present study was to investigate the genetic diversity of S. aureus recovered from the healthy horses in Iran according to coa gene.

Methods: Sample collected from the nasal cavities of 274 healthy horses from central and northwest part of Iran and the presence of S. aureus were examined. Then, variable region of the coa gene was amplified by PCR on S. aureus isolates. The amplicons were then digested with restriction enzyme HaeIII and the restriction fragments were resolved by agarose gel electrophoresis.

Results: From the 274 samples, 24 isolates (8.75%) were S. aureus and all 24 isolates have coa gene. The genotyping results showed 7 distinct RFLP patterns, designated as P1 to P7. One isolate (4.16%) recovered from northwest part of Iran (Maragheh) has one double PCR product in coa gene PCR and designed as P4 by RFLP. Tow isolates characterized by 700 and 850 bp and designed as P2 and P3 (8.3%), respectively, were just identified in central part of Iran (Yazd). The rest of isolates (95.8%) have a single PCR product with dominant pattern of 980 bp, isolated from several parts of Iran and designed as P1 (37.5%).

Conclusion: The results of the study suggest that the nasal cavity of healthy horses from different regions of Iran could be a reservoir of several genetic variants of S. aureus, with implications in public health that need to be more investigate.

Keywords: PCR-RFLP, coa gene, Healthy horse, Iran

P794: **Detection of pathogens which are responsible for mastitis in dairy cattle and determination of the best antibiotic against that in husbandries around the Ardabil city**

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Background and Aim: In current study, 100 milk samples from (30 -72 month years old) cattle in husbandries around the Ardabil with clinical signs (such as Redness, pain, discharge and clots in milk) were tested. In many cases, failure to identify the precise cause of mastitis pathogens cause to non-specific treatment; furthermore the Antibiotic Resistance is enhanced. The aim of this study is identification of most prevalent pathogen of mastitis and the best antibiotic for it.

Methods: as mentioned 110 milk samples were collected. Then samples were centrifuged. After that, one loop of pellet was taken& cultured on Mc.Conkey and Blood agar medium. Plates were incubated at 37ºc for 24hrs. The growth of bacteria was analyzed after incubation period. The bacteria from plates were observed macroscopically and also microscopically. Catalane, oxidase tests and gram stain were conducted to differentiate the family of bacteria. Also differential tests were performed for detection of species. then antibiotic sensitivity test with diffusion method was performed by vancomycine, penicillin, Sulfamethoxazole-trimethoprim, ceftriaxone ,tetracyclin, lincomycin, amoxicillin and ampicillin against most common pathogen

Results: From 100 samples, 22 samples were negative and 28, 5, 8, 22 and 15 samples were determined as Streptococcus. agalactiae, Staphylococcus .aureus, Streptococcus dysgalactiae, yeast and E.coli respectively. S.agalactiae (28%), S.dysgalactiae (8%) and S.aureus (5%) cause mastitis in dairy cattle respectively. Yeast (22%) and E .coli (15%)cause mastitis. the antibiotic sensitivity test defined 89.86 of S. agalactie isolated from bovine mastitis were susceptible to vancomycine and peniciline, 79.23% to Sulfamethoxazole-trimethoprim 72.45% to lincomycin and tetracyclin and 58.67% to ceftriaxone. On the other hand, there was not any sensitivity to ampicillin and amoxicillin.

Conclusion: This study shown that S.agalactiae is the main pathogen responsible for mastitis. Also, environmental pathogens such as Escherichia coli may be considered as the second factor. By performing correct management on the herds and use the best antibiotic it is possible to control mastitis caused by pathogen and environmental bacteria

Keywords: Mastitis, antibiotic resistance, Streptococcus, Staphylococcus, E.coli.

P795: **Identification of fungal flora in broiler hatcheries in Mazandaran province**

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Background and Aim: Microorganism contamination in hatchery has a serious impact on the viability and quality of chicks as well as on the overall growth performance of chickens. One of the most microbial problems in hatcheries is fungal contamination. This study was conducted to investigate the fungal contamination of air and the surface of equipment and facilities in hatcheries.

Methods: The study was carried out in 25 commercial broiler hatcheries located in Mazandaran province, during April 2012 to May 2013. The samples were collected from different locations in hatcheries such as: setters, hatchers, gas room, grade room, etc. SDA medium was used for the isolation of the fungi by open-plate techniques. Period of exposure of the plates was 30 minutes. All plates were incubated at 30° C aerobically for 7 days.

Results: Thirty-five species representing 27 genera were identified. The most prevalent genera were Cladosporium, Penicillium, and Aspergillus. Isolations increased in early spring and summer.

Conclusion: In spite of the regular disinfection in commercial hatcheries, fungal contamination was found in different parts. Statistical analysis of data is ongoing.

Keywords: Hatchery, Contamination, Chicks, Mazandaran.

P796: **survey of secretion of Brucella abortus vaccine strain RB-51 in cattles milk by Polymerase Chain Reaction**

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Background and Aim: Brucellosis is a zoonotic, chronic and infectious disease, which is caused by bacteria of genus Brucella. Important component involve in eradication of brucellosis is vaccination of calves or cows with Brucella abortus vaccine strain RB-51. Brucella spp. localize in the supramammary lymph nodes and mammary glands of infected animals and excrete in their milk. The purpose of this study was investigated secretion of brucella abortus vaccine strain RB-51 in cattles milk with direct PCR.

Methods: In this research, 60 milk sample collected from cattles in different villages of Kurdestan province. DNA was extracted for all milk samples directly. In order to detect the Brucella abortus (biovars 1,2 and 4) PCR carried out using B. a and IS711 primers on all DNAs, and in order to detect Brucella abortus vaccine strain RB-51, primers specific to the WboA gene were used.

Results: Of 60 milk samples, in 9 samples Brucella abortus (biovars 1,2 and 4) were detected. However, brucella abortus vaccine strain RB-51 were not detected in any samples.

Conclusion: Polymerase Chain Reaction is a rapid and sensitive method for detection of Brucella spp. in samples. Given that two months had passed since the vaccination of cattles, in any samples, vaccine strain RB-51 was not detected but may be less than 2 months after vaccination, we confirmed the presence of these bacteria in milk. As regards few studies in this area have been carried out, more comprehensive studies on the duration of secretion of vaccine strain RB-51 into cows milk, are needed.

Keywords: Brucella; cattle; milk; vaccine; RB-51, PCR

P797: **Detection of Brucella in goats milk by Polymerase Chain Reaction and comparision with Milk Ring Test**

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Background and Aim: Brucellosis is a contagious bacterial infection of livestock and continues to be of great health concern and economic importance worldwide, especially in developing countries. Man is infected by animals brucellosis through direct or indirect by ingestion of animal products as after drinking raw milk and/or eating unpasteurized milk products. The objective of this study was to detection of Brucella in goats milk by PCR and comparison it with MRT.

Methods: In this research, 30 milk samples collected from suspected brucellosis goats. Milk Ring Test carried out on all milk samples then DNA were extracted directly and primers B4 and B5 was used for PCR and detection of Brucella spp. in milk samples.

Results: Out of 30 milk samples, seven samples were positive according to the Milk Ring Test and 12 milk samples were positive by PCR. Six samples were just positive in PCR while were negative by using MRT and 1 sample were just positive in MRT while were negative by using PCR.

Conclusion: Based on the results of our study, PCR test for detection of brucella in milk samples from goats is more sensitive compare to Milk Ring Test, and PCR can be a useful tool for the detection of Brucella in goats milk samples.

Keywords: Brucella; Goat; Milk; MRT; PCR.

P798: **Evaluation of some hygienic parameters in Poultry Feedstuffs**

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Background and Aim: Animal Feedstuffs may serve as a carrier for a wide variety of microorganisms. The primary mode of inoculation of Feedstuffs materials is the transference of soil by wind, rain, or insects to standing crops. Some of the microorganisms are adapted to the desiccated and relatively nutrient poor conditions in soil and survive in similar niches on growing crops. Gastrointestinal pathogens can also introduced into the food chain by animals defecating in the farm environment or by fertilization of crops with manures. The present study constitutes a survey to obtain data for moisture, total bacteria counts, yeast and mould counts of feeds collected from Feedstuffs. In parallel, the presence of salmonella was tested in feed samples of vegetable origin collected from the feed industry. These data will serve as an essential source of information to the traceability A concept, developing Good Agricultural Practice (GAP) programmes, and also as an important prerequisite to the establishment of Hazard Analysis and Critical Control Point (HACCP) systems for the animal production sector in Iran.

Methods: 65 samples of feeds were collected from the storage areas of poultry farms .These samples consisted of 65 feeds (maize grain, 16; barley grain, 18; wheat grain, 19; cottonseed meal, 12). All samples were analyzed for moisture, total bacterial count, yeast and mould counts. In all cases, sampling of feeds was performed according to Institute of Standards and Industrial Research of Iran methods(ISIRI, 1810).

Results: Among the feeds examined, cottonseed meal was microbiologically best, and maize grain was worst. This may reflect the moisture content of the feeds, which was lowest for cottonseed meal and highest for maize. Barley grain, wheat grain, cottonseed meal, had high bacteria count (4.8–5.2 log10 cfu/g), and maize grain the highest yeast and mould count (5 log10 cfu/g) and moisture (12%). one of 65 (1.5%) feeds were positive for Salmonella spp.

Conclusion: Although the presence of salmonella is associated with animal products, such as foods or feeds, there is appreciable evidence to suggest that feed materials of plant origin are potential sources of Salmonella in animal production. The information from the present study will contribute to appropriate measures taken by the Iran State, to cope with EU legislation on food safety, to develop traceability systems and to establish a HACCP system as well as GAP programme for Iran.

Keywords: Poultry Feedstuffs, hygienic parameters, bacterial, yeast and mould count

P799: **Isolation and Identification of Lactate producing bacteria from the rumen of Mehraban sheep**

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Background and Aim: The rumen is a complex, open ecosystem which is inhabited by a diverse, dense, and competitive microbial population (Whitford et al.,1998). Host ruminants can provide the most stable environment in their rumen by different physiological mechanisms however microbial fermentation in the rumen can be rendered an activity outside of animal body (Alipour,2010). One of this microbial populations are lactate producing bacteria which consume readily fermentable carbohydrates and produce VFA (Volatile Fatty Acids) and lactic acid (Hernandez et al.,2007). There are two particularly outstanding practical reasons for interest in the lactic acid bacteria of ruminants. The first concern is lactic acidosis, especially in cattle and sheep. The second area of acute interest in the lactic acid bacteria of ruminants arises from claims that, in some cases, addition of lactic acid bacteria to the diet may increase the health and well-being of young animals, including lambs and calves (Steward,1992). The aim of this research is to isolate and identify the lactate producing bacteria in the rumen of Mehraban sheep.

Methods: The samples of rumen fluid were collected from three fistulated sheep and cultured on the broth of m-BA medium and Basal Medium 10 as reported by Al Jassim (2009). Thirteen bacterial isolates were grouped by Gram morphology. The 16S rRNA region of each isolate was amplified by PCR (Polymerase Chain Reaction) with the universal primers 16S rRNAfor: AGAGTTTGATCCTGGCTCAG and 16S rRNArev: ACGGCTACCTTGTTACGACTT (Lane,1992). Based on RFLP (Restriction Fragment Length Polymorphism) analysis of the PCR amplified 16S rRNA, twelve of isolated bacteria were chosen. The identity of these isolates was later confirmed by comparative DNA sequence analysis of the 16S rRNA gene.

Results: Three of the isolates were closely related to other previously characterized rumen bacteria, including Streptococcus macedonicus, Streptococcus luteciae and Enterococcus faecalis.

Conclusion: The percentage similarity amongst all strains was 99%, confirming the identification of the Mehraban sheep isolates.

Keywords: Streptococcus macedonicus,Streptococcus luteciae,Enterococcus faecalis,PCR,RFLP,sheep,rumen

P800: **Zahraei-Ziaei Vertical Double Antibiotic Synergy Test (ZZ-VDAST), a new approach for evaluating double antibiotic synergy**

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Background and Aim: It is well known that unprincipled and unfounded use of antimicrobial compounds may result in unintended and unpleasant consequences in the treatment of infectious diseases. Resistance to the synergism, new antibiotic antagonisms, empirical therapy in severely ill patient, and world health, etc. are the reasons emphasis on the necessity of antimicrobial concomitant usage. This study intends to introduce a new approach with additional benefits for evaluating antimicrobial interactions.

Methods: This study is based on laboratory data obtained from evaluation of effective factor on a method development. The combinations of penicillin G plus gentamicin and erythromycin plus clindamycin were compared using two methods including ZZ-VDAST and a reference method with structural similarity (disk diffusion synergy). Staphylococcus aureus ATCC 25923 and Escherichia coli ATCC 25922 were used as inoculum.

Results: Based on both zone average and incidence table, ZZ-VDAST, compared to the reference method, demonstrated an anticipated synergy and antagonism on both bacteria. Using ZZ-VDAST, the zone diameters in both synergistic and antagonistic situations were significantly ($p<0.05$) different in the form of enhanced and weakened effects.

Conclusion: On the base of the results of this study, we concluded that ZZVDAST could be employed as an efficient method in evaluation of antimicrobial combination. Further investigations are required to demonstrate the efficacy of this methodology on other bacteria.

Keywords: Zahraei-Ziaei ZZVDAST Antibiotic Synergy Combination

P801: **In vitro study of the effect of Alternaria alternata extract on myelin basic protein (MBP) pulsed dendritic cells and induced T cells response**

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Background and Aim: Multiple sclerosis(MS) is an autoimmune disease with impairment in function of CNS, Meanwhile macrophages and dendritic cells (DC) can cause inflammation and damage to The myelin of nerve cells by realizing of ROS and other harmful substances when these cells get matured. We investigated the effect of Alternaria alternata extract on maturation of MoDc (Monocyte-Derived Dendritic Cell),, Tcell responses, phagocytic and T cell stimulation activity of DC pulsed with MBP as a laboratory model of MS.

Methods: For this study Plastic adherent monocytes were cultured with granulocyte –macrophage colony stimulating factor (GM-CSF) and Interlekin-4 for converting these cells to modc and pulsed with MBP and matured in the presence of monocyte-conditioned medium (MCM) in control group and MCM+Alternariaalternata extract in treatment groups. phenotypical analysis and Phagocytic activity of DC were evaluated and also Tcell shifting proliferation responses were investigated.

Results: We found that, expression of CD14 decreased and CD83, HLA-DR increased in treatment groups in comparison with control groups. The amount production of IL-10 overcame IL-12 and in Tcell the production of cytokines; IL-17 and IFN-? decreased but in contrast IL-4 decreased. Phagocytic activity in treatment groups decreased significantly in compare with control group. Meanwhile, DC couldn't stimulate Tcell proliferation. These effects escalated with increasing of doze from 50 to100 (mg/ml).

Conclusion: Characterized in end, Alternaria alternata extract can cause maturation of MBP-pulsed modc and skewing of T lymphocyte toward Th2 also Alternariaalternata extract due to decreasing of phagocytic activity of modc -pulsed with MBP and no effect on Tcell proliferation can provide new strategies in immunotraphyof multiple sclerosis.

Keywords: Alternaria alternate, extract fungus, Myelin Basic Protein (MBP), Dendritic cell, Maturation, lymphocyte T

P802: **evaluation of virulence properties and comparison of invasiveness among Listeria monocytogenes isolatesd from clinical and food sources**

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Background and Aim: Listeria monocytogenes is an opportunistic intracellular pathogen that has become an important cause of human infections world wild. This bacterium is causative agent of listeriosis. Disease incidence is dependent on variety of factors including the presence of virulence factors, dose and general health and immune status of the host. More recently, different reliable and reproducible tests based on continuous cell lines (eg. HeLa and Caco-2) have been developed to distinguish between pathogenic and non-pathogenic Listeria Spp. The purpose of this study was to evaluate the virulence properties and invasiveness of Listeria monocytogenes strains isolated from food and clinical sources

Methods: This study comprised 14 strains of Listeria monocytogenes (9 clinical, 4 food and a conrol ATCC strain). To examine invasiveness of Listeria monocytogenes, HeLa cell line (cell culture method) was used. After infecting HeLa cells with prepared suspension of bacteria , qualitative and quantitative (Invasion Index) invasion properties of strains from two different sources (clinical and food) were evaluated.

Results: All isolates were invasive in HeLa cell culture method observed by light microscope after Giemsa staining. Invasion Indexes were in the range between 0.001 and 0.005. only two clinical strains had Invasion Index 0.006 and 0.007(both of them isolated from placental samples). Invasion Index of control strain was also 0.005.

Conclusion: It seems that for detection of correlation between virulence (severity of invasiveness) and source of infection with Listeria monocytogenes, more studies with much more strains will be necessary.beside that, PCR detection of known virulence genes has the potential to gain additional insight into their pathogenic potential. In authors previous study PCR was done for two main virulence genes and all of isolates were positive for those genes that is in agreement with cell culture resulte in this research.

Keywords: Listeria monocytogenese, virulence, food, clinical sources

P803: **Antimicrobial effect of hydro-extract of Pistacia atlantica on bacteria in invitro**

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Background and Aim: Infection diseases as a major cause of mortality make world width problem, therefore there is a huge attempt to deal with this issue. New antimicrobial agents design based on high antibactericidal potential, high specificity and low cross-reaction. Nowadays, antibacterial resistant microorganisms are fourth cause of mortality throughout the world and global effort to approach with it has been continued. Herbal remedy as an ancient treatment is considerable. Previously, herbal extract were exploited to treat infections such as; Aloa vera, Thymus vulgaris, Allium sativum. This study is aimed to investigate the effect of Pistacia atlantica on bacteria and compare its effect with routine antibiotics.

Methods: Sample size was determined 30 numbers per each bacteria by STATCALC software (EPI-Info). hydro-extract prepared from Pistacia atlantica that collected from Zagros mountains. Disck diffusion and well embedding method recruited to investigate antibacterial effect. MIC test done for hydro-extract and antibiotics. Statistic analysis done by Square Chi test, t independent test, t-test and fisher test.

Results: MIC of Pistacia atlantica extract were 163µg/ml, 104.16µg/ml and 204.67µg/ml for E.coli, Pseudomonas aeruginosa and Staphylococcus aureus, respectively. These funding shown inhibition effect of this herbal extract but it indicate no affect on H.pylori.

Conclusion: Funding indicate inhibit effect of hydro-extract on recruited bacteria except H.pylori thereby, it is suggested that phenol/chloroform-extracion could be have inhibit affect on H.pylori.

Keywords: H.pylori, Staphylococcus aureus, Pseudomonas aeruginosa, E.coli, Pistacia atlantica, Hydro-extraction

P804: **E-test antibiotic susceptibility of Pseudomonas aeruginosa strains isolated from hospital acquired infections of Imam Khomeini hospital, Ilam, Iran**

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Background and Aim: Pseudomonas aeruginosa is an opportunistic Gram-negative non-fermenting bacillus that belongs to the family Pseudomonadaceae. P.aeruginosa has minimal nutrition requirements, which contribute to its broad ecological adaptability and distribution. Therefore, the organism is common in the environment including soil, water, humans, animals, plants, sewage, and hospitals. It is important opportunistic pathogenic humans that can cause infections in the respiratory tract, blood, urinary tract, ear, skin and soft tissues, eye, central nervous system, heart, bone and joint and gastrointestinal tract. Also, infection with P.aeruginosa is a major health problem for immune-compromised patients and individuals with cystic fibrosis. Antibiotic resistance is a major feature of infections that the widespread use and misuse of antibiotics is one of the main reasons for the emergence of resistance. Therefore, the aim of this study is determine the antimicrobial susceptibility of P. aeruginosa clinical strains isolated from Ilam hospitals by E-test method.

Methods: A total of 30 clinical isolates of P. aeruginosa were obtained from medical centers and hospitals in Ilam city. Its, then, through culture on the sitrimaed medium, ability production of green pigment and other biochemical tests were identified. Then, antibiotic susceptibility was determined by using E-test method to antibiotics commonly used in medical centers and hospitals (e.g., Amikacin, Amoxicillin, Cefepime, Ceftazidime, Ceftriaxone, Ciprofloxacin, Gentamicin, Meropenem, Nitrofurantoin, Piperacillin, Tetracycline, Ticarcillin, Tobramycin, Sulfamethoxazole-Trimethoprim, Vancomycin and Oxacillin).

Results: In this study, 100% of isolates were resistant to Amoxicillin. In contrast, all isolates were 100% sensitive to the antibiotics Amikacin, Cefepime, Meropenem and Tobramycin. Sensitivity to the antibiotics Gentamicin, Trimethoprim, Piperacillin, Ceftazidime and Ciprofloxacin were 93.4, 93.4, 93.4, 90.1 and 90.1 percent, respectively. Also, resistance to Ceftriaxone and three antibiotics Nitrofurantoin, Tetracycline and Ticarcillin were 76.9, 66.6 percent, respectively.

Conclusion: Based on the results, the highest sensitivity were determined to the antibiotics Amikacin, Cefepime, Meropenem, Tobramycin, Gentamicin, Piperacillin and Trimethoprim and least sensitive were to Amoxicillin. Thus, given the increasing antibiotic resistance in hospitals are necessary committees rational drug prescribing in hospitals are cooperating closely with the infection control committee.

Keywords: Pseudomonas aeruginosa, antimicrobial susceptibility, E-test

P805: **Prevalence of antimicrobial resistance of bacteria from ICUs unit at Namazi hospital in Shiraz, Iran, 2008-2009**

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Background and Aim: Introduction: Nosocomial infections are an important health-care problem for their morbidity and mortality. Incidence of nosocomial infection, especially in intensive care unit (ICU) ward, is high. More than 20% of all nosocomial infections are acquired in ICUs. Patients with severe underlying disorders requiring intensive care are particularly prone to nosocomial infections caused by opportunistic pathogens or hospital strains of bacteria. Considering the perpetual existence of resistant bacteria in the ICU as well as the hindrance they cause against therapy, it is necessary to gain comprehensive knowledge of these bacteria and their respective pattern of antibiotic resistance profile.

Methods: Methods: This cross-sectional, descriptive study was conducted to determine the pattern of antibiotic resistance for common bacteria in ICUs ward of Namazi Hospital, Shiraz, Iran from October 2008 through October 2009. A total number of 1095 samples were administered to the microbiological laboratory of the Shiraz University of Medical Sciences. The isolates from the cultures were identified using standard microbiological and biochemical laboratory techniques. Once the bacteria were distinguished, their sensitivity for antibiotics was studied. The disk diffusion test was used for the measurement of antimicrobial susceptibility according to the Clinical and Laboratory Standards Institute recommendations.

Results: Results: In this study, specimens of 1095 patients were studied. 1198 isolates were identified from these samples. The microbiological isolates were included: 569 (47.5%) from Gram-positive bacteria, 629 (52.5%) from Gram-negative bacteria. Among 629 gram- negative isolates, Pseudomonas was the most prevalent one, 160 (25.5%), E.coli 133 (21%) and Acinetobacter spp. 115 (18%) were the next common pathogens isolated. Our findings indicate the greatest resistances of Gram negative bacteria belong to cefixime (2%). Among 569 gram- positive isolates, Staphylococcus spp. 506 (89%) was the most prevalent bacterium isolated. In total, of the 569 Gram- positive Isolates, 1.2% were resistant to erythromycin. 1.16% of the isolates were resistant to three or more antibiotics and were designated as multidrug resistant (MDR).

Conclusion: Conclusion: Nosocomial infections are associated with a great deal of morbidity, mortality and increased financial burden. Intensive care is a risk factor for the emergence of antibiotic resistant bacteria. Knowledge of emerging pathogens and resistance profile is essential for treatment against nocosomial infections.

Keywords: Keywords: Antimicrobial resistance, ICUs unit, Namazi hospital, Shiraz, Iran

P806: **Frequency of Herpes Viruses 6 infection in febrile children in an Iranian referral Hospital**

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Background and Aim: Polymerase chain reaction (PCR) tests for virus in blood cells as well as saliva are frequently positive in persons with past infection, reinfection with new strains of virus, or latency with or without repeated reactivation of Herpes Viruses 6 (HHV6) virus. The aim of our study was to determine the frequency of HHV-6 infections in children ≤ 2 years who had initial diagnosis of fever during an evaluation in the pediatric emergency department of the children medical center, an Iranian referral hospital by polymerase chain reaction (PCR).

Methods: In all children, the clinical characteristics noted at the initial evaluation as well as demographic and laboratory finding were obtained.

Results: Among 150 patients, 91 boys and 59 girls admitted to the pediatric emergency department and HHV6 was found in 49 patients (33%) including 14 girls (29%) and 35 boys (71%). Rashes was seen in 14/49 (29%) of cases with positive HHV-6, while 35 cases without rashes had positive PCR test (71%). Seizures were found in 78/150 of patients (52%). There was not significant association between seizures and positive HHV-6 results (43% in patients without seizure and 57% in cases who developed seizure).

Conclusion: Although standard PCR on different samples including blood cannot discriminate between latent and active HHV-6 infection, in our study nearly one third of patients mainly children younger than 1 year had HHV-6 infection.

Keywords: Herpes Viruses 6, Polymerase chain reaction, Children

P807: **Assessment of antibacterial properties of Ziziphora tenuior L. essential oil**

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Background and Aim: Due to emerging of resistance of microorganisms to antibiotics, investigations for novel antimicrobial agents have always been one of the major preoccupations of the medical society. Traditional medicine systems have played an important role during human evolution and development. Today, a number of medical herbs around the world have been studied for their medicinal activities. Amongst the several herbal medicine used as a medicine. This study was carried out to the determine antibacterial activites and chemiacal compositions of essential oil of Ziziphora tenuior obtained from the hydro_distillation.

Methods: The essential oil of Ziziphora tenuior was extracted by hydro-distillation technique using Clevenger apparatus and was analyzed by capillary GC and GC/MS method. Anti bacterial properties of the essential oil on three pathogenic bacteria were determined by using disk diffusion methods. The determination of minimal inhibitory concentration (MIC) and Minimum Bactericidal Concentration (MBC) were evaluated for this essential oil. The antimicrobial potent of of Ziziphora tenuior essential oil was compared with commercial antibiotics. Each experiment was done three times and collecteddata were analyzed by SPSS .

Results: The essential oil showed the maximum anti bacterial effect on Shigella flexeneri and the minimu Streptococcus pneumoniae and salmonella typhi. Twenty-three componets were identified in Ziziphora tenuior oil that pulegone(90.63%) as major componets in essential oil.

Conclusion: Our data clearly indicated that the Ziziphora tenuior extract have antibacterial properties against,Shigella flexeneri ,salmonella typhi and Streptococcus pneumonia comparable with standard antibiotics.

Keywords: Ziziphora tenuior, essential oil pulegone, anti-bacterial properties,MIC,MBC.

P808: **Helicobacter pylori iceA1/2 genotypes could be implicated as a risk biomarker in the high-incidence areas of gastric cancer in Iran**

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Background and Aim: Helicobacter pylori infects human stomach causing chronic inflammation, which can lead to peptic ulcers and gastric cancer. Iran is a country with high prevalence of H.pylori infection. The incidence of gastric cancer in the North and North-Western districts has been reported considerably high, while it is low in the Southern regions. The aims of the present study were to assess i) genotypes of iceA (a virulence gene of H.pylori) in strains obtained from different provinces of Iran and ii) differences in distribution of these genotypes between the high- and low-incidence of gastric cancer areas in Iran.

Methods: A total of 138 H. pylori isolates were obtained from biopsy cultures in 10 provinces of Iran. Presence of the iceA1 and iceA2 genes was investigated using PCR amplification. The hierarchical analyses of molecular variance (AMOVA) and Mantel tests by GenAlEx and Chi-square ($\chi 2$) and Fisher's exact tests by SPSS were used in this study.

Results: AMOVA for the iceA1 and iceA1/2 genotypes found significant levels of genetic differentiation among populations ($P < 0.05$). The prevalence of iceA1/2 gene (but not iceA1 gene) was significantly higher among H. pylori isolates from the high- than low-incidence areas of gastric cancer in Iran ($P < 0.005$). The results of Mantel's test showed a low correlation between the genetic and geographic distances for the iceA1 and iceA1/2 genes among 10 districts of Iran ($r= 0.098$ and 0.074, respectively, $P < 0.05$).

Conclusion: Presence of iceA1 and iceA2 simultaneously in the same strain could be considered as a risk biomarker in the high-incidence areas of gastric cancer in Iran.

Keywords: Helicobacter pylori, Gastric cancer, iceA, Iran

P809: **Isolated agents of acute bacterial meningitis and their antimicrobial profiles in Namazi Hospital, Shiraz, Iran, 2012-2013**

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Background and Aim: Meningitis receives a high level of medical, public health and media attention because of its rapid onset and high level of morbidity and mortality. To diagnose bacterial meningitis, cerebrospinal fluid (CSF) examination is mandatory. CSF culture remains the gold standard for the diagnosis of bacterial meningitis; and it is obligatory to obtain the in vitro susceptibility of the causative microorganism to rationalize treatment. Acute bacterial meningitis is caused by a variety of microorganisms. The prevalence of these organisms varies from place to place, by age and season.

Methods: During a 24 months period from 2012-2013, 2230 samples of CSF from patients with suspected meningitis were examined. All CSF samples were collected by the attending physicians with meticulous aseptic technique and followed for isolation of the bacteria in culture media (blood agar, chocolate agar and enrichment broth thioglycolate) incubated for 48 hours in humid air plus 5-10% CO2. Bacterial isolates were then detected by standard bacteriologic procedures and antibiotic susceptibility tests were done for the isolates by using disk diffusion method in accordance with the CLSI guidelines.

Results: Of 2230 samples,255 (11.4%) were detected as culture positive. More common bacterial isolates were as follow: Coagulase-negative staphylococci (CoNS) (52.1%), Streptococcus spp. (7.8%), Staphylococcus aureus, Escherichia coli and Acinetobacter spp. each with (5.8%), followed by, Klebsiella spp. (5.4%), Pseudomonas and Enterococci species (3.9%). Gram positive isolates were mostly resistant to erythromycin (70.2%), cephalexin (61.7%), lincomycin (58.4%) and trimethoprim-sulphametoxazol (56.7%). They were highly susceptible to vancomycin (91.5%), chloramphenicol (75.2%), cloxacillin (65.1%) and gentamycin (53.9%). Among the recovered Gram negative isolates,high resistance were detected against tetracycline (98.4%), cefixime (95.3%) and cephalexin (92.1%) and the most sensitivity prevalence were detected against gentamycin (65.6%), chloramphenicol (42.1%) and ciprofloxacin (40.6%).

Conclusion: Although the countenance of bacterial meningitis has changed substantially over the past 15 years, this disease still causes significant morbidity and mortality, particularly in developing countries. Based on these data the empirical treatment strategy for meningitis differs. Empirical antibiotic therapy should be adjusted to local drug resistance patterns and clinical subgroups to help rationalize treatment.

Keywords: cerebrospinal fluid (CSF) , meningitis , Antibiogram

P810: **Antimicrobial effects of crude extracts of mangrove plant (Avicennia marina) against bacterial and fungal strains**

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Background and Aim: Mangroves are various plants that grow in the intertidal zones of saline coastal in the tropical and subtropical areas. Mangroves have been widely used for therapeutic proposes from the past. Currently, indiscriminate consumption of antibiotics has caused emerge of resistance in pathogens. The aim of our research is to find new compounds from crude oil extracts of mangrove plant (Avicennia marina) that have been antibacterial effects against some of bacteria and fungi including E. coli, Bacillus subtilis, Pseudomonas aeruginosa, Staphylococcus aureus, Aspergillus niger and Candida albicans.

Methods: Considering in this objective, fresh leaves and roots of Avicennia marina were collected from mangrove forest in saline coastal sediment habitats in Gheshm Island, located in southern Iran. The collected samples after transferring laboratory were washed 3 times with running tap water and then, twice in sterile water before drying at ambient room conditions. Following, the dried tissues were ground by a mortar and pestle until being formed powdered. To extract of whole bioactive compounds 5 g of powdered materials was macerated in 100 ml of methanol/water, ethanol/water and ethyl acetate in the ratio of 80: 20 for each solvent extracts. After 24 hours the samples were separated from debris using whatman´s filter paper and concentrated by rotary evaporator to obtain a certain amounts of each solvent extract. The extracts subsequently were water diluted and followed; all dilutions were assay for antimicrobial activity against chosen bacteria and fungi based agar well diffusion technique and zone of inhibition (mm) was determined after 24 h incubation at 37° C. Tetracycline (30µg/ml) was selected as positive control and all samples were compared with it.

Results: Methanolic extract indicated to be efficient on gram positive bacteria, namely Staphylococcus aureus and Bacillus cereus but no activity observed against other microorganisms. Effectiveness of ethanolic extract was closely similar to methanolic one. Interestingly, ethyle acetate strongly affected on growth of Aspergillus niger and Candida albicans inn addition to Staphylococcus aureus and Bacillus cereus.

Conclusion: Although this research provided a preliminary knowledge about bioactivity of natural compounds extracted from mangrove plants in Iran, it is the first report of antimicrobial property for indigenous Avicennia marina. Thus, it is suggested that bioactive compounds of mangrove plants could be good therapeutic effects against infectious diseases.

Keywords: Antimicrobial activity, Bioactive compounds, mangrove

P811: **Isolation of bacteria from foot wounds in diabetes patients**

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Background and Aim: Foot wounds infections are highly common in diabetes people that could lead to amputation. The aim of this retrospective study was to isolate of causative agents from foot ulcers and to assess their comparative in-vitro susceptibility to some antibiotics in Tabriz city.

Methods: Standard microbiological techniques used for isolation of bacteria from ulcers and antimicrobial susceptibility tests were performed in accordance to diffusion method.

Results: Of 41 patients with foot wounds,38 of them having cultures that were positive.Staphylococcus aurous was as the most common isolate , being recovered from 37 % causes. It was followed by Enterococci spp. (17%), Ecoli (16%),non enterococcal streptococci spp (9%). S .epidermidis (8%).From Pseudomonas aeruginos , klebsiella spp. Entrobacter spp. and Citrobacter spp. Proteus spp. bacteria , only one isolate was identified. Out of 14 S.aurous isolates 5 strains were found resistant to methicillin.

Conclusion: Vancomycin was the most effective agent against Gram positive bacteria and Imipenem , Ceftriaxone, Ciprofloxacin, and Rifampin were the most effective agents to Gram negative organisms.

Keywords: Diabetes , Foot wounds infections , etiologic agents.

P812: **SCCmec typing of methicillin-resistant Staphylococcus aureus strains isolated from clinical isolates of Children Medical Center Hospital, Tehran, Iran**

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Background and Aim: Methicillin-resistant Staphylococcus aureus (MRSA) is an important human pathogen and responsible of many nosocomial infections and it has the highest potential of morbidity and mortality in burn patients. The aim of this study was to SCCmec typing of MRSA isolated obtained from clinical isolates of Children Medical Center Hospital, Tehran, Iran.

Methods: In total, 133 S. aureus isolates collected from hospitalized patients (range: from 1 day to 10 years). S. aureus isolates were recognized based on morphology, Gram stain, mannitol salt agar fermentation, catalase and coagulase test. Isolates growing on the Mueller- Hinton agar plates containing 2 mg/mL oxacillin with 4% NaCl were considered to be MRSA strains. DNA was extracted from MRSA strains using phenol/chloroform and Lysostaphin enzyme. All MRSA isolates were confirmed by the presence of the mecA gene by PCR. Multiplex PCR assay for SCCmec typing was performed on all of MRSA isolates.

Results: Among of 133 S. aureus isolates, 70 (52.6%) were MSSA and 63 (47.4%) were MRSA. Among the 63 MRSA strains, 36 strains (57%) had single SCCmec type including type I (n=2, 3.2%), type II (n=4, 6.3%), type III (n=19, 30.2%), type IVa (n=2, 3.2%), type IVc (n=3, 4.8%), type IVd (n=2, 3.2%) and type V (n=4, 6.3%). However, 25 strains (40%) had two types including types III + IVa (n=15, 23.8%), III + IVb (n=3, 4.8%), III + IVc (n=4, 6.3%), IVb + IVd (n=3, 4.8%) and 3.2% of MRSA strains (n=2) were not typeable. Among the 63 MRSA strains, there were 48 (76.2%) Health-care-associated MRSA (HA-MRSA) and 15 (23.8%) community-associated MRSA (CA-MRSA).

Conclusion: In our study, 47.4% of strains were MRSA and majority of them were HA-MRSA. Type III (30.2%) was the highest single SCCmec type followed by combination of type III and IVa (23.8%).

Keywords: SCCmec typing, MRSA, Multiplex PCR.

P813: **Antibiotic resistant of Acinetobacter baummanii isolates with genotyping method in Tehran hospitals**

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Background and Aim: Acinetobacter baumannii is the most important of nosocomial infections especially in ICU. Furthermore increasing resistance to antibiotics caused of inability to treatment of infections by these bacteria. This study designed to the aim of antibiotic resistance of A. baumannii isolates using genotyping method in Tehran hospitals

Methods: This study was performed on 500 clinical samples that they were collected from different hospitals in Tehran. A.baumannii isolated were identified by using cultured and biochemical test in lab . After determining drug resistance by the disk diffusion method using CLSI guidelines, PFGE was performed on all isolates. Finally, the results of each genetically patterns were compared with results of antibiogram.

Results: In this research that was found 100 isolate were A. baumannii & 30 of theme were other species of Acinetobacter. Dendrogram plot of genotyping of isolates revealed that 7 different genetically pattern were exsist and this pattern were named from A_E. The results revealed all of these patterns were resistant to cefepime) Cpm), ceftazidime (ca), so they are not good choice for treatment. In addition was determined that the only F pattern was sensitive to norfloxacin (Nx) and only A pattern was sensitive to ciprofloxacin and only G pattern was sensitive ofloxacin (Ox) and amikacin these results indicate that most strains of these bacteria are resistant to antibiotics. Finally it was found that most isolates are sensetive to ampicillin_sulbactam & tobramycin, so these drugs are good choice to treatment.

Conclusion: This study showed that different type of isolated from different hospitals have same antimicrobial resistance pattern so make a detailed plan cuse perevent spread of this bacteria. In addition most of isolate sensitive to ampicilin- sulbactam & tobramicin and these drugs can be used to treat infections caused by bacteria.

Keywords: Antibiotic resistant, Genotyping, Acinetobacter baumannii

P814: **Dissemination of the Metallo-β-Lactamase Gene blaimp1 among Pseudomonas aeruginosa isolates in Imam Reza Hospital of Mashhad, Iran**

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Background and Aim: Carbapenems exhibit potent anti-pseudomonal activity, but The clinical utility of carbapenems is under threat with the emergence of acquired carbapenemases, particularly Ambler class B metallo-β-lactamases (MBLs). To identify the metallo-B-lactamases (MBLs) prevalence in Imam Reza hospital of Mashhad, Iran, clinical isolates of Pseudomonas aeruginosa with a reduced susceptibility to imipenem (IPM) was subjected to PCR analyses with specific primer to blaimp1

Methods: A total of 113 P. aeruginosa isolates were collected from hospitalized patients in Imam Reza hospital of Mashhad, Iran, between Jun 2011 and Nov 2012. Disk diffusion method with disks containing imipenem and blank+10ml EDTA (0.5 M) was used for determination of the presence of metallo- b-lactamases Following total DNA extraction with simple boiling method, the strains were evaluated for the presence of MBL-encoding gene by PCR amplification with specific primer for blaimp1.

Results: From a total of 131 isolates of P. aeruginosa, 61 isolates were found to be resistant to Imipenem. From these isolates, 53 (86%) strains of P. aeruginosa isolates were determined to be MBL producers by phenotypic method. By PCR, the IMP1 gene were detected in 4 (7.5%) isolates.

Conclusion: This study showed a high prevalence of and MBL-producing strains of P. aeruginosa isolated and a low prevalence of the Metallo-β-Lactamase Gene blaimp1 among Pseudomonas aeruginosa isolates in Imam Reza Hospital of Mashhad, Iran

Keywords: Imipenem, Metallo-β-Lactamase Genes, Pseudomonas aeruginosa

P815: **Molecular characterization on class 1 integrons in E. coli isolated from urinary tract infection in Rasht, Iran**

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Background and Aim: An integron is a genetic unit that includes the determinants of the components of a site-specific recombination system capable of capturing and mobilizing genes that are contained in mobile elements called gene cassettes. The development of antimicrobial agents has been a key achievement of modern medicine. However, their overuse has led to an increasing incidence of infections due to antibiotic-resistant microorganisms. Transference of resistance determinants by integrons is one of the important factors that can contribute to the increase in multi-resistant bacteria. The aim of the present study was to recognize class 1 integrons in E. coli isolated from urinary tract infection in Rasht.

Methods: All isolates (33 strains) were examined for resistance to routine antimicrobial agents by standard disk diffusion method. Genomic DNA was extracted from all of them. Detection of class 1 integrons was performed by PCR. To determine whether the strains carried integron, the conserved regions of integron-encoded integrase gene intI1 were amplified.

Results: Our results showed that all E.coli isolates identified as multi-drug resistant (MDR). PCR results revealed that 97% strains contain Int1 gene. The detected fragment was about 500 bp.

Conclusion: Because these strains can pass the resistant gene to other clinical strains, the quick detection of these strains in clinical laboratories and their control is very important.

Keywords: Integron, gene cassette, resistance, antibiotic

P816: **Prevalence and Characterization of Vancomycin Intermediate and Resistance Staphylococcus aureus isolates from Tehran, Iran**

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Background and Aim: Background and aims: Staphylococcus aureus causes severe infection in not only in the hospital setting but also in the community. Vancomycin is mostly the antibiotic of choice for the treatment of infections caused by methicillin-resistant Staphylococcus aureus (MRSA). In recent years, strains of S. aureus with reduced susceptibility to vancomycin (VISA) were reported from various parts of the world. The aims of this study were to determine the prevalence and some genetics characteristics of VISA strains in clinical isolates of S.aureus from hospitals of Tehran.

Methods: Methods: 415 isolates of S. aureus were collected from clinical specimens in selected hospitals in Tehran, Iran. Then, they were evaluated by disk diffusion, agar screening and E-test to determine their resistance to vancomycin. The isolated VISA and VRSA strains were then confirmed with genetic analysis by the evaluation of mecA and vanA genes, and all VISA isolates exanimated for determine the SCCmec and agr types

Results: Results: Our data indicated that 54% of isolated S. aureus strains were resistant to methicillin. Agar screening tests revealed that 17.5% (n=73) of 415 S.aureus produced colony. In E-test, VISA phenotype was determined in 3.1% (n=12) of isolates. Only one VRSA strain was detected (MIC 24 µg/ml), this strain was negative for vanA gene. Most of our VISA isolates belonged to agr group II. Moreover, their SCCmec types belonged to types I and III.

Conclusion: Conclusions: The presence of S.aureus with intermediate resistance to vancomycin is alarming and it is crucial to screen for vancomycin resistance prior to antibiotic therapy. While, development of VISA phenotype in MSSA strains may result in increased tolerance to other classes of antibiotics are used to treatment of staphylococcal infection.

Keywords: Staphylococcus aureus, VISA, VRSA and MRSA

P817: **Prevalence of Extensively Drug Resistant Helicobacter pylori strains among patients with different gastric disorders in Iran, a perspective study during 2010 – 2011**

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Background and Aim: Background: Emergence of multidrug resistant (MDR) strains of Helicobacter pylori (H. pylori) with increased values of minimal inhibitory concentrations (MICs) for most commonly prescribed antibiotics have made treatment decisions difficult. Aim: The purpose of this study was to determine frequency of MDR and extensively drug resistant (XDR) H. pylori strains and their MIC values against different antimicrobial agents in Iran - in 2010-2011.

Methods: MIC values of rifampicin, ciprofloxacin, levofloxacin, amoxicillin, clarithromycin, erythromycin, metronidazole and tetracycline were determined for 111 strains out of 197 dyspeptic patients by agar dilution method. After 72 h of incubation, the MIC of each antibiotic was determined. H. pylori isolates resistant to at least three families of potentially effective antimicrobial agents were considered as isolates with MDR phenotype. The isolates that were resistant to all but one or two classes of potentially effective antimicrobial agents were categorized as XDR.

Results: The primary resistance rates were 61.3% for metronidazole, 15.3% for amoxicillin and 14.4% for rifampicin. The rate of resistance to the other antimicrobials was as follows: macrolides (erythromycin and clarithromycin) 32.4%, and quinolones (levofloxacin and ciprofloxacin) 30.8%. Among the resistant strains, double drug resistance and MDR phenotype was detected in 22.6% (19/84) and 34.5% (29/84), respectively which is the highest rate that has been reported until now. XDR strains of H. pylori was detected in 11 (37.9%) out of 29 examined MDR strains. Only one of these strains remained susceptible to metronidazole.

Conclusion: These data collectively showed a great concern about emergence of XDR H. pylori strains and suggest tetracycline containing compounds as most likely successful regimens for treatment of infections by these strains. Governmental policy towards antibiotic misuse is essential to overcome increasing resistance to these and newer antimicrobials against this bacterium.

Keywords: Antimicrobial Susceptibility, MDR, XDR, MIC, Helicobacter pylori, Iran.

P818: **Simultaneous specific detection of Streptococcus pneumoniae, Haemophilus influenzae and Mycoplasma pneumoniae in sputum samples from patients with suspected influenza by Multiplex-PCR**

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Background and Aim: Streptococcus pneumoniae, Haemophilus influenza and Mycoplasma pneumoniae are the most common cause in bacterial pneumonia. Also these agents can cause bacterial super infection in patients with influenza. Aim of this study was Simultaneous specific detection of Streptococcus pneumoniae, Haemophilus influenza and Mycoplasma pneumoniae in sputum samples from patients with suspected influenza by Multiplex-PCR.

Methods: In this study, 170 sputum samples in patients with suspected influenza with age from 3 months to 70 years, received the Influenza Reference Laboratory – Tehran Medical university were tested by Multiplex PCR. Amplified DNA fragments size was 394 bp for Streptococcus pneumoniae, 199 bp for Haemophilus influenzae and 416 bp for Mycoplasma pneumoniae.

Results: of all 170 samples, 30 samples were positive for Streptococcus pneumoniae and Haemophilus influenza. Of the 30 positive samples, 27 samples (15/8 %) and 3 samples (1/7 %) were positive for S. pneumoniae, Haemophilus influenza respectively.

Conclusion: This study showed that Multiplex-PCR able to diagnosis desired bacteria in short time and so this molecular method can use as complementary technique especially when the results of gram stain, culture or serological test are negative.

Keywords: Detection, Streptococcus pneumoniae, Haemophilus influenza, Mycoplasma pneumoniae, Multiplex-PCR

P819: **Comparsion of the effect of Lactobacillus Acidophilus Suppository and Metronidazol Vaginal Tablet on Bacterial Vaginosis**

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Background and Aim: Bacterial vaginosis is characterized by an imbalance in vaginal natural discharges due to an overgrowth of anaerobic bacteria as well as a concomitant decrease in the number of lactobacilli and increase in vaginal PH to more than 4.5. Bacterial vaginosis increases the risk of pelvic inflamatory disease, postoperative infection following hysterectomy, cervix abnormal cytology and preterm birth. Microbial resistance and a growing tendency to move toward "natural therapies" have led to increase interests in non-antibiotic treatments for bacterial vaginosis. This study aimed to compare the effects of Lactobacillus acidophilus suppository and Metronidazol vaginal tablet on treatment of bacterial vaginosis.

Methods: In a double-blind, paralled randomized clinical trial, 40 out of 300 participants were included in the study from an out-patient private gynaecology clinic in Tabriz, Iran. The participants were randomly allocated to two treatment groups of Lactobacilli suppository and Metronidazol vaginal tablet.

Results: The cure rate for the Metronidazol group and the Lactobacilli supository group were 100 and 75 per cents, respectively.

Conclusion: According to the results of this study, Metronidazol was more effective in treatment of bacterial vaginosis than the Lactobacilli suppository. A combination of these medications are suggested for resistant to treatment cases.

Keywords: Bacterial Vaginosis, Lactobacillus, Metronidazol

P820: **The association of interleukin-18 promoter polymorphisms and serum levels with duodenal ulcer and its correlation with bacterial CagA and VacA virulence factors**

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Background and Aim: Interleukin (IL)-18, a proinflammatory cytokine, induces T-helper 1 differentiation and cytotoxic T-lymphocyte functions, both of which have been proposed in the pathogenesis of duodenal ulcer. In this study we analyzed the impact of IL-18 promoter polymorphisms on IL-18 serum levels in H. pylori-infected duodenal ulcer (DU) patients and healthy asymptomatic (AS) carriers. We also aimed to determine the association of the H. pylori virulence factors, cytotoxin-associated gene A (CagA) and vacuolating cytotoxin A (VacA) antibodies with serum concentrations of IL-18 as to elucidate any correlation between them.

Methods: A total of 67 DU patients, 74 healthy AS subjects as the control group enrolled in this study. Serum concentrations of IL-18 were determined by ELISA method. Patients sera were tested by Western blot method to determine the presence of serum antibodies to bacterial virulence antigens-p120 (CagA), p95 (VacA). Genotyping of IL-18 promoter polymorphisms at positions -137 G/C and -607 C/A were performed by Allele-Specific Primer (ASP) - polymerase chain reaction (PCR) protocol.

Results:: Our study revealed that serum IL-18 levels are positively influenced by CagA-positive H. pylori strains, so that maximum levels of IL-18 (331±69 pg/ml) were detected in DU patients with the CagA+ phenotype, regardless of the presence of the anti-VacA antibody. Regarding IL-18 promoter polymorphisms, the AA genotype at position ?607 C/A was found significantly higher in controls than in patients (OR=0.322, 95% CI: 0.121-0.911, P = 0.032). Furthermore, frequencies of A allele at ?607 position in patients reduced once compared to control group, demonstrating the protective role of -607 A variant for DU (OR=0.607, 95% CI: 0.374-0.986, P = 0.043).

Conclusion: Our study also revealed that subjects with the -137G/-607C haplotype have an increased risk of duodenal ulcer development probably due to higher IL-18 induction in these individuals. Overall, our study demonstrated that IL-18 -607 C variant was linked with higher levels of serum IL-18 and increased risk of DU. Moreover, our findings indicated that serum concentrations of IL-18 were influenced by CagA factor, independent of the VacA status, suggesting that high levels of IL-18 in CagA positive subjects are predisposed to susceptibility to DU.

Keywords: Helicobacter pylori, interleukin (IL)-18, CagA and VacA, promoter

P821: **Circulating levels of interleukin (IL)-12 and IL-13 in Helicobacter pylori-infected patients, and their associations with bacterial CagA and VacA virulence factors**

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Background and Aim: This study aimed to determine the association of the H. pylori virulence factors, cytotoxin-associated gene A (CagA) and vacuolating cytotoxin A (VacA) antibodies with serum levels of interleukin (IL)-12 and IL-13 in H. pylori-infected duodenal ulcer (DU) patients, H. pylori-infected asymptomatic (AS) carriers as to elucidate any correlation between them.

Methods: A total of 67 DU patients, 48 AS individuals and 26 healthy H. pylori-negative subjects enrolled in this study. Serum concentrations of IL-12 and IL-13 were determined by ELISA method. Patients sera were tested by Western blot method to determine the presence of serum antibodies to bacterial virulence antigens p120 (CagA), p95 (VacA). Serum concentrations of IL-12 and IL-13 were compared in nine groups including four AS phenotypes (CagA+VacA+, CagA+VacA-, CagA-VacA+, CagA-VacA-) and four DU phenotypes (CagA+VacA+, CagA+VacA-, CagA-VacA+, CagA-VacA-) and one control group.

Results: The results revealed that DU patients positive for the CagA, independently of the anti-VacA antibody status, showed drastically elevated levels of IL-12 (251±43 pg/ml), when compared with other groups ($p=0.0001$). No significant difference was found between groups regarding levels of IL-13 ($p>0.05$).

Conclusion: Our findings indicated that in DU group the serum concentrations of IL-12 but not of IL-13 were influenced by the bacterial CagA, independently of the VacA status, suggesting that high IL-12 levels may contribute to DU susceptibility in CagA positive individuals. These findings can possibly be considered to improve the predictive or prognostic values of inflammatory cytokines for DU, and also to design possible novel therapeutic approaches.

Keywords: Helicobacter pylori, IL-12, IL-13, CagA, VacA

P822: **Levels of interleukin-(IL)-12p40 are markedly increased in brucellosis among patients with specific IL-12B genotypes**

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Background and Aim: Brucellosis remains a major zoonosis worldwide. Brucella antigens induce the production of T-helper 1 (Th1) cytokines such as interleukin- 12 (IL-12) in humans. We aimed to investigate the association of two single nucleotide polymorphisms (SNPs) in the gene encoding the IL-12p40 cytokine (IL12B) with brucellosis, and to examine the functionality of these SNPs through measuring serum levels of IL-12p40.

Methods: We genotyped IL-12B gene rs3212227, A>C; rs6887695 G>C polymorphisms in a case-control study on a totally of 281 subjects including 153 patients with active brucellosis and 128 healthy controls, using RFLP, and serum IL-12p40 levels were assessed by ELISA.

Results: The rs3212227 minor allele (C) and homozygote genotype (CC) were more frequent in controls compared with brucellosis patients (p=0.006, OR=0.608, 95%CI=0.429-0.861for the C allele; p= 0.024, OR = 0.443, 95% CI: 0.218-0.900 for the CC genotype). Comparison of IL12B genotypes and serum levels of the IL-12p40 revealed that rs3212227 AA genotype, with higher frequency in patients than in controls, was associated with increased levels of the cytokine (p=0.0001). Furthermore, the distribution of haplotype and genotype combinations in our study suggested that rs3212227C/rs6887695C haplotype or CC/GC or CC/CC genotype combinations may protect controls against Brucella infection by contributing to a functional downregulation of the serum IL-12p40 production in vivo, as shown by ELISA ($p<0.05$).

Conclusion: Overall, our study demonstrated that rs3212227 A variant was associated with higher levels of serum IL-12p40, and could possibly contribute to an inherited predisposition to brucellosis.

Keywords: brucellosis, interleukin (IL)-12p40, gene polymorphism

P823: **Survey Frequency of Verotoxigenic Escherichia coli in Raw-Milk at Ilam City**

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Background and Aim: Some E.coli strains produce cytotoxins that it first described by Konowalchuk and coworkers.This toxin have cytopathic effect on Vero cells and identified as Verotoxin. Verotoxin producers Eschrichia coli (VTEC) belong to varity O-serotypes and have relation to Hemolytic Uremia Syndrome (HVS) and Hemoragic syndrome (HS). There is little information about prevalence and distribution of VTEC in Iran. This study performs for resolve frequency of VTEC in Ilam province. In this survey frequency of raw-milk contamination to verotoxigenic E.coli was investigated in one year Period. Samples consist 375 raw-milk that collected from city shops and 122 raw-milk samples that collected from industrial animal husbandry. For Isolation of Verotoxigenic E.coli, we used of extraction with polymixin method and non fermentation of Sorbitol and Agglutination with O157: H7 Antiserum method for detection of O157: H7 sertypes.

Methods: روش نمونه‌گيري در اين پژوهش روش نمونه‌گيري از شيرهاي سطح ايلام روش خوشه‌اي دو مرحله‌اي (two stage cluster sampling) نمونه‌گيري به عمل آمد. (random) مي‌باشد . ولي از نمونه‌هاي موجود در گاوداري‌ها به صورت تصادفي نمونه‌ها پس از خريد به ظرف استريل منتقل شده، اطلاعات آنها ثبت و در شرايط استريل به آزمايشگاه ميكروب‌شناسي دانشگاه ايلام جامعه آماري جامعه آماري در اين پژوهش عبارت است از شيرهاي خامي كه در سطح شهر توزيع .منتقل و مورد آزمايش قرار گرفتند مي‌شوند و همچنين نمونه شيرهايي كه در گاوداري‌ها گرفته مي‌شوند. در اين مطالعه اندازه نمونه عبارت است از 497 نمونه كه شامل 375 نمونه شير توزيع شده در سطح شهر و 122 نمونه كه از گاوداري‌ها تهيه شده است.

Results: از 375 نمونه (75/45%) شير خام تهيه شده از سطح شهر ايلام تعداد 7 نمونه (1/85%) آلوده و از تعداد 122 نمونه جدول 1 نشان مي‌دهد كه به .(24/55%) تهيه شده از گاوداري‌ها تعداد 2 نمونه (1/64%) آلوده به اشريشيا كلي وروتوكسيژنيك بودند طور كلي با فاصله اطمينان 98% براي برآورد آلودگي به اشريشيا كلي وروتوكسيژنيك و مقايسه فواصل اطمينان ميزان آلودگي بين شهرهاي تهيه شده از سطح شهر ايلام و گاوداري‌ها اختلاف معني‌داري وجود ندارد در حالي كه از نظر فصول مختلف تفاوت جدا شده از نمونه‌هاي مورد مطالعه VTEC توليد وروتوكسين به وسيله‌ي سويه‌هاي .معني‌داري از نظر آلودگي مشاهده نمي‌شود به VT2 به وسيله 5 سويه (55/50%) و VT1جدا شده از شيرهاي خام، VTEC آزمايشات خنثي‌سازي نشان داد كه از ميان 9 سويه بود VT2 و VT1 وسيله 3 سويه (33/3%) توليد مي‌شود. يك سويه (11/11%) نيز توليد كننده.

Conclusion: نتايج تحقيق در مجموع نشان داد كه آلودگي نمونه‌هاي شير به اشريشيا كلي وروتوكسيژنيك در نمونه‌هاي شيرهايي كه از گاو داري صنعتي تهيه مي‌شوند نسبت به آنهايي كه در سطح شهر به فروش مي‌رسند اختلاف معني داري ندارند و همچنين سروتايپ O157: H7 در اين شهرستان از شيوع پاييني برخوردار است. از طرفي عدم آشنايي كامل دامداران با اصول علمي استريليزاسيون و عدم استريل ظروف انتقال در فاصله بين دو بار شيردوشي و انتقال شير به محل فروش به طوريكه در اين فاصله آنها فقط به شستشوي ساده ظروف با آب اكتفا مي‌كنند اين امر مي‌تواند يكي از دلايل آلودگي و اختلاف احتمالي ميزان آلودگي شيرهاي مورد آزمايش باشد.

Keywords: Raw Milk ،Verotoxin ،Escherichia coil

P824: **Frequency of bacteria causing Urinary Tract Infections (UTI) and their antimicrobial resistance patterns among pediatrics patients in the West of Iran during 2007-2009**

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Background and Aim: Urinary Tract infections are among the most common infections among infants and neonates. The aim of the current study was to evaluate the frequency of bacteria causing UTI and their relevant drug resistance patterns among infants and neonates hospitalized in Ilam province, west of Iran during 2007-2009

Methods:: A total of 220 cases of UTI were enrolled in this cross-sectional retrospective study. A standard checklist was used for demographic and clinical data to be collected from their health records. Data was then analysed using SPSS version 17.0.

Results: More than two third (64.8%) of the cases were female. E. coli (44.5%), Klebsiella (18.6%), Enterobacter (15 %) and Staphylococcus spp. (12.7%) were the most common microorganisms isolated from UTIs, respectively. High rates of resistance to tetracycline, ampicillin, and nalidixice acid were observed among these isolates

Conclusion: Similar to other studies, E.coli was the most common bacteria causing UTI and showed a high rate of resistance against most of the antimicrobial agents. The use of appropriate antibiotics against UTIs and establishment of annual surveillance programs on determining the antimicrobial sensitivity of bacteria to routinely used antibiotics can be helpful for physicians in choosing a proper treatment in patients suffering from UTI and also to reduce the complications related to serious UTI.

Keywords: Urinary Tract Infection, Drug resistance, infant and neonates, Iran

P825: **Phenotypic and genotypic characteristics of aminoglycosides resistance among MethicillinResistant Staphylococcus aureus (MRSA) isolated from different era in west Iran.**

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Background and Aim: Aminoglycosides play an important role in the treatment of staphylococcal infections, despite the emerging widespread resistance among Staphylococcus.

Methods: To determine the prevalence of aminoglycoside resistance and aminoglycoside modifying enzyme (AME) genes among infected patients at different hospitals in the West of Iran, we tested 100 Staphylococcus aureus as determined by the disk diffusion method and multiplex PCR. The minimum inhibitory concentration of gentamicin for each isolate was determined by E- test. .

Results: All methicillin-resistant staphylococci were mecA-positive by PCR. Of the 100 isolates, 20 % were resistance to to gentamicin, kanamicin, tobramicin and amikacin. The most prevalent AME genes included aph(2)-Ia (83.3%) followed by aph(3)-IIIa (44.4%) and aph(2)-I(33.3%) and ant(4)-Ia (33.3%), respectively. All aminoglycoside-resistant staphylococci contained at least one AME gene. The coexistence of two or three AME genes was detected in most gentamicin-resistant isolates.

Conclusion: These results suggest an alarming rate of aminoglycoside resistance in this test location in Tehran, Iran. Continued surveillance at the genotypic and phenotypic levels, and adherence to welldesigned antibiotic and infection-control policies are necessary to limit the spread of antimicrobial resistance.

Keywords: Antibiotic resistance, Aminoglycosides, MRSA,Western of Iran.

P826: **Phenotypic and genotypic characteristics of tetracycline resistant Acinetobacter baumannii isolates, isolated from nosocomial infections at Tehran Hospitals**

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Background and Aim: To date, the most important genes responsible for tetracycline resistance among A. baumannii isolates have been identified as Tet A and Tet B. This study was carried out to determine the rate of resistance to tetracycline, related antibiotics and mechanisms of resistance.

Methods: A total of 100 A. baumannii isolates were recovered during 2010-2011 from patients in different hospitals of Tehran, Iran. Antimicrobial susceptibility to tetracycline, minocycline, doxycycline and tigecycline was evaluated by E-test. PCR of the Tet A and Tet B genes was performed using specific primers, after which the isolates were subjected to REP-PCR to identify the major genotypes.

Results: Resistance and intermediate rates, to tetracycline, minocycline and doxicycline were 89% (MIC50 32µg/ml, MIC90 512µg/ml), 35% (MIC50 16µg/ml, MIC90 32µg/ml) and 25% (MIC50 16µg/ml, MIC90 32µg/ml), respectively. Frequencies of Tet B and Tet A genes and coexistence of Tet A and Tet B among the isolates resistant against tetracycline were 87.6%, 2.2% and 1.1%, respectively. REP-PCR showed the five clusters A, B, C, D and E with distribution rates of 40% (n=40/100), 30% (n=30/100), 10% (n= 10/100), 5% (n=5/100) and 5% (n=5/100) respectively.

Conclusion: It seems that Tet A and Tet B genes play an important role in the induction of resistance against tetracycline used in this study. It is suggested that further studies focus on other antimicrobial drugs and combinations in order to achieve a successful therapy against MDR A. baumannii strains in Iran.

Keywords: Tet A, Tet B, tetracycline resistance, REP-PCR

P827: **Prevalence of serum antibodies to (Toxoplasma gondii) in patients in some clinical laboratories province**

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Background and Aim: research about Toxoplasmosis in som of cities in guilan.

Methods:: In this descriptive and analytical study of 800 sera of individuals referred to the medical diagnostic laboratories in Gilan in 1391 randomly selected, and the use of ELISA IgG and IgM antibodies against Toxoplasmosis was measured

Results:: Based on the results of total 800 of the 301 men and 499 were female, 261 (62/32 percent) had IgG antibody against a Toxoplasmosis of IgM antibody were negative. Population of men (9/87percent) and women (22/75%) of IgG antibody were detected. Using chi square test, no significant relationship between antibody prevalence with age, sex, location job and no history of contact with pets

Conclusion: The results confirm that the necessary test to diagnose toxoplasmosis. Health awareness, people can also reduce the risk of toxoplasmosis.

Keywords: Toxoplasma gondii, ELISA, IgG, IgM

P828: **Evaluate multiple drug resistance (MDR) and pan drug resistance (PDR) in P. aeruginosa using the Agar Disk Diffusion Test at Taleghani and Golestan hospitals Ahwaz**

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Background and Aim: Since Pseudomonas aeruginosa is the highly resistant to many antimicrobial agents, in addition to the intrinsic resistance of bacteria to many antibiotics, it quickly becomes resistant to drug during treatment. Generally, these bacteria use multiple drug resistance mechanisms such as producing Beta-lactamase Enzyme, outer membrane permeability and expression of efflux pumps to develop resistance to drugs during treatment. Today, it has been shown that antibiotic resistance in bacteria is the result of, Synergism between membrane permeability and multi drug resistance Efflux pumps. Objective: The aim of this study is to evaluate multiple drug resistance (MDR) and pan drug resistance (PDR) in P. aeruginosa using the Agar Disk Diffusion Test at Taleghani and Golestan hospitals Ahwaz.

Methods: One hundreds fifty P. aeruginosa were isolated from burn and wound infections and Antibiotic resistance among them was evaluated using Agar Disk Diffusion Test.

Results: In this study, a total of 150 samples were taken, 66% of which showed multiple drug resistance (MDR) and 0.67% of those showed pan drug resistance (PDR) to these antibiotics. 61.33% of wound samples and 70.66% of burn samples were positive for MDR. Of the burn samples 1.33% resistance to PDR, while wound samples showed no resistance.

Conclusion:: In this study, finds that MDR of P.aeruginosa is increasing. Checking MDR in burn and wound samples, showed that this resistance in burn infections is more than wound infections. PDR was observed in burn samples; whereas, it was not observed in wound infections.

Keywords: Multiple drug resistance, pan drug resistance, Pseudomonas aeruginosa, Agar Disk Diffusion Test

P829: **Assessment of the frequency of Staphylococcus aureus carriers and its antibiotic susceptibility in nursing, midwifery and paramedical students (input in the years 2011 and 2012) at the School of Nursin**

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Background and Aim: Nasal colonization with community acquired methicillin resistant Staphylococcus aureus (CA-MRSA) is being increasingly reported, specially in places where people are in close contact . In this study we investigated the frequency of MRSA colonization and their antibiotic susceptibility in students of nursing, midwifery and paramedical in Rafsanjan University of Medical Sciences, South-East of Iran.

Methods: Two handred nasal swabs were collected from students of nursing, midwifery and paramedical that had no risk factors for colonization by S. aureus. The speciments were cultured for isolation of S. aureus by standard methods. Antimicrobial susceptibility testing was performand by disk diffusion method according to the clinical and Laboratory standards Institute (CLSI) gudelines. For evaluation of the frequncy of erythromycin induced clindamycin resistance, disk approxmation test (D test) was applied.

Results: Among 200 of studied cases the frequency of 5% nasal carriers for Staphylococcus aureus is determined.six (60%) of the 10 S. aureus isolates were MRSA strains. 50% of MRSA and 25% of methicillin susceptible S. aureus(MSSA)was resistant to clindamycin.four of the 6 strains of MRSA and 1 of the MSSA strains were resistant to erythromycin and D test was positive in 50% of cases.

Conclusion: Nasal carrier student of the resistant variants of Staphylococcus aureus are always a serious threat to their own and others. We conclude that the rate of colonization by methicillin resistant S. aureus is high in studied cases and Regarding the frequency of induced resistance to clindamycin in MRSA cases, screening Staphylococcus aureus isolates in this regard, seems to be essential.

Keywords: methicilin resistant Staphilococcus aureus (CA-MRSA) , nasal carriers, Antibiotic resistance pattern, inducible resistant

P830: **Comparison antimicrobial effect of Tamarix hispida extract on planktonic and biofilm form of Escherichiacoli.**

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Background and Aim: Biofilm formation is a reason for drug resistance in bacteria. So inhibition biofilms is a good way to overcome microbial drug resistance. In this study antimicrobial activity of Tamarix hispida extract on planktonic and biofilm form of Escherichia coli was evaluated.

Methods: Tamarix hispida extracts was prepared by methanol and ethanol in maceration technique. The antimicrobial activity of extracts was evaluated by disk diffusion method. Microbroth dilution was used to determine MIC value. The effectiveness of extracts on biofilm formation was examined by plate microtitre method. Three concentration containing 5, 10 and 20 mg/ml of each extract was applied in this test.

Results: Inhibition zone of both extracts in disk diffusion test was obtained 8 millimeters. MIC value for methanolic extract was 2.5mg/ml and for ethanolic extract was 1.25mg/ml. Each extract can prevent biofilm formation significantly. With decreasing concentration, inhibitory effect was reduced too. Ethanolic extract was more effective than methanolic one. Ethanolic extract with concentration of 20 mg/ml inhibited the biofilm formation 50%.

Conclusion: Based on this study alcoholic extract of Tamarix hispida can be a perfect choice for controlling Escherichia coli in planktonic and biofilm form.

Keywords: Tamarix hispida, Escherichia coli, drug resistance, biofilm.

P831: **Detection of Cytomegalovirus in positive CMV IgG samples by polymerase chain reaction (PCR)**

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Background and Aim: Cytomegalovirus (CMV) is an important and common cause of mortality and morbidity in immunocompromised patients. After primary infection with CMV the virus becomes latent in multiple organs and can later be reactivated during severe dysregulation of the immune system. A large population carry dormant virus and are thus at risk for reactivation. The purpose of this study is introduces a fast and accurate method for diagnosis of CMV-DNA and to better define the relationship between CMV-IgG serology and detection of CMV –DNA in serum samples

Methods: The gB oligonucleotide primer sets which amplify a fragment of the region that encodes the glycoprotein B were chosen. These primers amplify genome regions that are considered as a high degree of conservation of the epitope between the clinical isolates. Uniplex PCR with 257 bp fragment as PCR product was optimized. Sensitivity and specificity of the test was evaluated. 100 serum IgG positive have been collected from Noor Tehran laboratory. Then by using DNG method the DNA of specimens were been extracted. The optimized PCR test with specific primers of CMV was done for all of the samples.

Results: In the optimized PCR test, the DNA fragment of 257 bp was amplified. The test had a high sensitivity and specificity level. Among 100 patients with IgG positive serum, 5 cases (5 %) were positive for CMV-PCR test.

Conclusion: Our results showed that the percentage of IgG positive sera still positive test which confirms that there is CMV DNA in serum. PCR technique detects latent CMV DNA in these samples may be without active viral replication.

Keywords: Cytomegalovirus, PCR, positive CMV IgG

P832: **The prevalence of ESBL enzymes in Escherichia coli strains isolated**

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Background and Aim: The most common cause of broad-spectrum cephalosporin resistance in bacteria Escherichia coli (E.coli) is caused by ESBL-producing enzymes (ESBLs). in the past two decades, ESBLs have been reported in E. coli and enterobacteriases. These studies have been conducted to the prevalence of ESBLs producing enzymes in the E. coli.

Methods: In this study, 250 urine samples were collected from Ibn Sina Hospital in 1391 and 100 E. coli were identified using standard biochemical tests. Antibiotic susceptibility testing by the agar disk diffusion method and ESBLS produced using a combination of clinical phenotype, including disk and disk diffusion agar was determined by CLSI standardized.

Results: A total of 100 E. coli, 80 strains producing ESBLs were isolated using phenotypic tests.

Conclusion:: in E. coli is produced the beta - Lactamazes with capabilities hydrolyzed broad-spectrum cephalosporin, monobactam and penicillins. Producing ESBLs is related to the previous use of anti-microbial. Lactamase production has spread widely among countries.

Keywords: broad-spectrum beta-lactamase enzymes (ESBLs), Escherichia coli(E.coli) , disk diffusion

P833: **Isolation of Melittin from honey bee venom by Reverse Phase-HPLC technique**

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Background and Aim: Honey bee venom consisted of many ingredients including antibacterial peptides, enzymes, and chemical molecules. Melittin is one of Antimicrobial peptides (AMP) which is the main component of honey bee venom. It has broad antimicrobial activity against many bacteria. In this study honey bee venom purified using Reverse Phase-HPLC technique and melittin isolated for subsequent analysis.

Methods: Honey bee venom collected by electrical stimulation. The quality of venom examined by SDS-PAGE. Melittin purified from bee venom using a linear gradient of acetonitryl applied to a C18 column. The resultant fractions monitored at 214 and 280nm, collected, and lyophilized for further antibacterial assay.

Results: 20 major and minor fractions were detected. SDS-PAGE confirmed that melittin was the major fraction at 2.8kDa. Melittin was removed from the column approximately at 47.5 to 52.5 minutes. This period corresponds to the percentage of acetonitrile from 42.5 to 47.5 percent.

Conclusion: Antibacterial peptides from various sources including plants, animals, crustaceans, arthropods, mollusks and invertebrates and vertebrates have been isolated by chromatography techniques such as HPLC, FPLC and etc. Lyophilized melittin fraction intended for determination of minimum inhibitory concentration against standard species of bacteria.

Keywords: Honey bee, Venom, Melittin, RP-HPLC

P834: **Antibacterial activity of melittin against bacteria involved in bacterial peritonitis**

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Background and Aim: Bacterial peritonitis is a nosocomial infection that occurs as the result of direct bacterial injury. During the past decades, morbidity and mortality of bacterial peritonitis have been reported from many hospitals around the world. Nowadays antibiotic-resistant bacteria seriously threaten human health due to the high incidence of these kinds of infections. Natural sources of antibiotics may be a good choice for future treatment regimens. Antimicrobial peptides (AMPs) have been isolated from many species including single-celled microorganisms, insects and other invertebrates, plants, amphibians, birds, fish, and mammals, including humans. AMPs have typically 12 to 100 amino acids, positively charged, and are amphiphilic molecules. Honey Bee venom is one of the important source of antimicrobial peptide, Melittin. The goal of this study was evaluation of in vitro antibacterial activity of isolated melittin against the main agents of bacterial peritonitis including Escherchia coli, Staphylococcus aureus, and Pseudomonas aeruginosa.

Methods: Melittin (purified from the previous study) concentration was estimated by BCA method. Antibacterial activity of melittin examined using Microdilution broth based on NCCLS guidelines with some modification. Escherchia coli ATCC 25922, Staphylococcus aureus ATCC25923, and Pseudomonas aeruginosa ATCC27853 used as standard strain for antibiogram. Briefly 100µg melittin serially diluted in muller hinton broth and subsequently 1.5 × 105 bacteria added to each well. The microplate incubated at 37°C for 16 to 20 hours. Minimum inhibitory concentration (MIC) considered as the latest well that was apparent by visual inspection. Minimum Bactericidal Concentration (MBC) was obtained by plating the wells in muller hinton agar and counting the subsequent colonies. MBC reported as concentration that 99.9% of bacteria were killed.

Results: MIC for Melittin against Escherchia coli, Staphylococcus aureus, and Pseudomonas aeruginosa were 0.39, 6.25, and 12.5 µg respectively. MBC were determined as 1.56, 25, and 50 µg respectively. The ratio of MIC/MBC for all three strains was 0.25 µg.

Conclusion: Nosocomial infections have always been a major clinical problem. One of the most common nosocomial infections is bacterial peritonitis during peritoneal dialysis. Like other bacterial infections, antibiotic resistance in bacterial peritonitis is not an exception and leads to significant morbidity and mortality. During the recent decades AMPs have been noted for treatment of bacterial infections. In this study, in vitro bacteriostatic and bactericidal activity of melittin examined against the agents of bacterial peritonitis. Based on the results Melittin molecules can have a destructive impact on all three types of the bacteria and amount of melittin for induction of growth inhibition and killing was comparable to conventional antibiotics.

Keywords: Melittin, Bacterial peritonitis, MIC, MBC

P835: **Oxacillin resistenance in coagulase negative staphylococci isolated from laryngoscope in Shahid Rajaei hospital, Qazvin.**

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3- departement of chemistry faculty of heath

Background and Aim: Background&Aim: Oxacillin is commonly used in the treatment of beta-lactamase resistant staphylococci which causing several clinical infections. In vitro routine test for oxacillin resistance may fail to detect due to presence of the mec(A) gene resulting in treatment failure.

Methods: Materials and Methods: twenty seven staphylococci isolates were subjected to routine antibiotic susceptibility testing including oxacilin(1µg) by kirby- bauer disk diffusion method. The oxacillin resistance was detected by agar dilution (4% NaCl + ox 6 µg / ml in M.H.A/24 h /33-350c) and then with PCR for mec (A) gene.

Results: Results: Intotal, 5 isolates (31.2%) were resistant to oxacillin. Finally, two coagulase negative staphylococci isolates(11.76%) carried mec (A) genes.

Conclusion: Conclusion: This study showed that handle of laryngoscope can be contaminated with resistant isolates. Considering the notable oxacillin resistant these isolates cleaning and use of appropriate disinfectant is necessary to decrease the spread of them by laryngoscope in the special wards of hospital.

Keywords: Keywords: Laryngoscope, staphylococci, D-test

P836: **Prevalence of the esp and asa1 genes in Enterococcus spp. isolated from vaginal swabs of women with spontaneous abortions.**

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Background and Aim: Enterococcus spp. are natural inhabitants of the gastrointestinal tract and the vagina in humans but has become increasingly important as hospital-acquired pathogens. Several virulence factors have been described in Enterococcus, for instance, aggregation substance, enterococci surface protein.Aggregation substance, encoded by asa1, which is carried on a plasmid, is a pheromone-inducible protein that enables the conjugative transfer of sex pheromone gene-containing plasmids through the clumping of one Enterococcus to another. The Enterococcus surface protein, encoded by the chromosomal esp which contributes to biofilm formation, and adhesion to eukaryotic cells. The aim of this study was to investigate the presence of esp and asa1 genes in Enterococcus isolates.

Methods: The Enterococcus strains isolated from 198 vaginal swabs from women with spontaneous abortion, were identified to the genus level with cultural and biochemical characteristics. Enterococci strains were further identified to the species level by biochemical tests. PCR assay was performed using specific primers of esp and asa1genes.

Results: 126 enterococci isolates were detected of all 198 vaginal swabs,(E. faecalis 65% and E. faecium 34%). According to PCR, 46 and 81 of 126 isolates (36.5% and 64.2%) were positive to esp and asa1genes, respectively.

Conclusion: The esp gene was detected in 31.1% of E. faecium isolates, against with the findings of other studies, which identified the esp gene in about 80% of E. faecium strains. In general, differences between the results of incidence of virulence genes obtained from this study and previous studies may be due to the source of sample collection. These finding suggested an effective control strategy of these bacteria in patients. This research will be continued by investigation of clonally spread of resistant strains by pulsed field gel electrophoresis.

Keywords: esp and asa1 genes, PCR, Enterococcus spp

P837: **Pathological study of experimental keratitis treated with propolis alcoholic extract in contaminated rabbits with Fusarium solani**

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Background and Aim: Propolis is a wax similar substance and bee byproduct. Antifungal properties of propolis have been identified in vitro condition.

Methods: In this study, the effect of ethanol extract of propolis in the treatment of experimental keratitis contaminated with Fusarium solani in rabbit was investigated. The samples of propolis were extracted using 60% ethanol. The experimental keratitis was created in 18 rabbits with suppressing of immune system and inoculating of yeast. The rabbits in six groups were classified and treated with glycerol (negative control), nystatin (positive control) and dilution concentrations of propolis alcoholic extract (1000 µg/ml, 500 µg/ml, 250 µg/ml and 125 µg/ml). The rabbits were euthanized and tissue samples were obtained from their eyes.

Results: After 21 days of treatment period, the keratitis symptoms were severed in control group. Experimental keratitis in rabbits inoculated with nystatin and 1000 µg/ml concentration of propolis alcohol extract was healed 14 and 21 days after treatment period respectively. The rabbits inoculated with a concentration of 500 µg/ml propolis alcoholic extract were achieved only partial improvement. The lesion of the cornea at 125 and 250 µg/ml concentrations didn't remove completely so that abundant pus in the tissue under normal epithelium were remained. In the nystatin group in compared to the treated group with 1000 µg/ml concentration of ethanol extract of propolis not show any difference.

Conclusion: The results were showed that propolis can be healed with 1000 µg/ml concentration completely. So the alcoholic extract of propolis can be a suitable alternative for medicines such as nystatin.

Keywords: experimental infection, keratitis, Fusarium solani, propolis alcoholic extract

P838: **Antibacterial effect of water extracts of Lepidium sativum (Alborz accession) on the bacteria P.aeruginosa PTCC 1430**

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Background and Aim: Since undesirable and side effects of chemical drugs and antibiotics, use of natural components especially medicinal plants is recently more interested in bacterial disease therapy.

Methods: In this research, anti-bacterial effects of water extracts of garden cress (Alborz accession) were investigated on P.aeruginosa PTCC 1430.In order to well diffusion and disk diffusion methods were used. Each plate inoculated with 100 µlculture of P.aeruginosa PTCC 1430whit 0.5 MC Farland concentration. Also 100 µl of water extract wasinfused to wells. In well diffusion method distilled water and tetracycline antibiotic 150 µg/µl were used as negative and positive control respectively. In disk diffusionmethod tetracycline was used as positive control. MIC test was performed to supplementary study, 1ml nutrient broth mediumcontaining 50 µlcultures of E.coliand various concentrations of Water extract (20, 40, 60, 80, 100, and 120)µl, were used. After 24 hrs C, Inhibition zone and MIC were evaluated.°incubation at 37

Results: For P.aeruginosa PTCC 1430 bacterium, inhibition zone was 5 mm in well diffusion method. Positive control inhibition zone was 12mm. Inhibition zone were 3mm and 9mm for garden cress water extract and positive control in disc diffusion method. Also, MIC was 40 µl.

Conclusion: As a result these experiments show the significant antibacterial effect of water extract Lepidium sativum(Alborz accession) on the bacteria P.aeruginosa PTCC 1430.

Keywords: Lepidiumsativum, antibacterial activity, well diffusion method, disc diffusion method, MIC, P.aeruginosa PTCC 1430.

P839: **Antibacterialeffect of water extracts of Lepidium sativum (Alborz accession) on the bacteria E.coli PTCC1399**

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Background and Aim: Since undesirable and side effects of chemical drugs and antibiotics, use of natural components especially medicinal plants is recently more interested in bacterial disease therapy.

Methods: In this research, anti-bacterial effects of water extracts of garden cress (Alborz accession)were investigated on E.coli PTCC1399.In order to well diffusion and disk diffusion methods were used. Each plate inoculated with 100 µlculture of E.coli PTCC1399whit 0.5 MC Farland concentration. Also 100 µl of water extract wasinfused to wells. In well diffusion method distilled water and tetracycline antibiotic 150 µg/µl were used as negative and positive control respectively. In disk diffusionmethod tetracycline was used as positive control. MIC test was performed to supplementary study, 1ml nutrient broth mediumcontaining 50 µlcultures of E.coliand various concentrations of Water extract (20, 40, 60, 80, C, Inhibition°100, and 120)µl,were used.After 24 hrs incubation at 37 zone and MIC were evaluated.

Results: For E.coli PTCC1399 bacterium, inhibition zone was 4 mm in well diffusion method. Positive control inhibition zone was 12mm. Inhibition zone were 1.3 and 8 mm for garden cress water extract and positive control in disc diffusion method. Also, MIC was 40µl.

Conclusion: As a result these experiments show the significant antibacterial effect of water extract Lepidium sativum(Alborz accession) on the bacteria E.coli PTCC1399.

Keywords: Lepidiumsativum, antibacterial activity, well diffusion method, disc diffusion method, MIC, E.coli PTCC1399.

P840: **Rapid detection of resistance to Rifabutin in Mycobacterium tuberculosis isolates**

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Background and Aim: Mutations in three main codons (516-526-531) of rpoB gene have the most relationship with drug resistant to rifabutin. In the present study an Allele Specific-PCR method by new primers was designed and results were compared with sequencing.

Methods: forty isolates Mycobacterium tuberculosis were used in the study. Specific primers were designed by MEGA-BLAST-IDT software for an Allele Specific PCR method. The primers could be detect the desired hot point in the gene from 3´end in 516-526-531 codons and could reconnoiter mutant state; Therefore the lack of formation of band in electrophoresis means that the strain was resistant. Some selected samples were sequenced and used as golden standard.

Results: From 40 clinical isolates of M. tuberculosis including 12 isolates was rifabutin resistant and 28 rifabutin susceptible strains. Mutations in one the three codons were detected in 10 strains. Susceptible strains have no any mutations in these codons. Results of sequencing were concordant with results of ASP method.

Conclusion: The results showed that Allele Specific-PCR was a rapid and simple method for fast detection of rifabutin resistance and recommended for routine work.

Keywords: Mycobacterium tuberculosis, resistance, Rifabutin, Allel Specific-PCR

P841: **Prevalence and antibiotic resistance pattern of Acinetobacter spp isolated from environment and in-patients of a teaching hospital in Isfahan, Iran**

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Background and Aim: Health care Associated Infections (HAI) caused by Acinetobacter spp have emerged in recent years and become a major problem in the health care settings. High level resistance to broad-spectrum antibiotics is the most important clinical aspect of this bacterium that increases morbidity and mortality rate in hospitalized patients. Unfortunately, in many routine clinical microbiology laboratories, Acinetobacter spp are not taken as an important pathogen and dismisses as contaminant. Acinetobacter spp have unique ability to survive in the hospital environments for long periods. This feature increases the rate of acquisition of infection of hospitalized patients by this high resistant bacterium. The aim of this study was determination of prevalence and antibiotic resistance pattern of Acinetobacter spp isolated from environment and hospitalized patients in a teaching hospital in Isfahan.

Methods: 1. Environmental samples During a three-month period, using the swab method, 40 samples were taken from different parts of the ICU and surgery wards of the hospital. All samples were examined for the presence of Acinetobacter spp. 2. Clinical specimens A total of 865 various clinical specimens including blood, CSF, wound, catheter, tracheal secretions, sputum, peritoneal fluid, urine, eye discharge, Broncho Alveolar Lavage (BAL), abscess, access, throat, cerebral shunt and tissue were surveyed for isolation of Acinetobacter spp. Identification was done by several phenotypic tests such as growth on MacConkey agar medium, gram stain, oxidase test, TSI, SIM, O-F medium containing 10% glucose and DNase. After isolation and identification, Antibiotic susceptibility test to 11 antibiotics was done by disk diffusion method for clinical and environmental isolates according to CLSI-2011 guidelines.

Results: In this study from 40 swab samples, 202 isolates were obtained that 109 isolates (53.96%) were belonging to gram positive bacilli and coccoci and 93 isolates (46.03%) were gram negative. The study showed in the culture of 52.5% samples Acinetobacter spp was grown. 21 Acinetobacter (10.96%) were isolated in this study. Most of the isolates were obtained from sink and bed of patients' room, respectively. All of isolates were Multi-Drug Resistance (MDR) and 18 isolates (85.71%) were extensively drug resistant (XDR) because of their resistance to carbapenems. Also from 865 clinical specimens 69 Acinetobacter spp (7.98%) were obtained by biochemical tests. Most of them were isolated from tracheal secretion (20.3%). In surveillance of antibiotic susceptibility test only 9 (13.04%) isolates were MDR and most of them (60/86.95%) isolates were XDR.

Conclusion: Our study showed increase of prevalence of Acinetobacter spp specially XDR-isolates in the environment and among hospitalized patients, thus treatment of infected patients has become a serious problem in the health care settings. Therefore infection control committee of any hospital should be performed serious measures for eradication of bacterium by removing of source of infection.

Keywords: Acinetobacter spp, prevalence, environment, drug resistance

P842: **Relation of babA2 genotype of Helicobacter pylori with peptic ulcer Disease in East Azerbaijan, Iran**

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Background and Aim: H. pylori is a microaerophilic, spiral shaped Gram-negative bacterium that efficiently colonizes the human stomach mucosa. Helicobacter pylori plays a causative role in the pathogenesis of various gastroduodenal diseases including gastritis, peptic ulcer, gastric cancer and mucosa-associated lymphoid tissue (MALT) lymphoma. The main bacterial virulence factors comprise adhesions (BabA, SabA, OipA, AlpA, AlpB and HopZ), the vacuolating cytotoxin VacA, and the products of the cag pathogenicity island (cag PAI). The blood group antigen binding adhesin (BabA), a 78-kDa outer membrane protein, encoded by the babA2gene, binds to Lewis b antigens and ABO antigen on gastric epithelial cells . There are two distinct babA alleles (babA1and babA2) and one highly homologous gene, babB, but only the babA2 allele is functionally active. The presence of babA2 is associated with duodenal ulcer disease and gastric cancer, and when found in conjunction with cagA and vacAs1 alleles, it leads to an even greater risk of developing more severe disease. The main aim of the present work was to study the associations of H pylori virulence factor (babA2) with PU and chronic gastritis in East Azerbaijan patients.

Methods: In total, 95 strains from 95 patients, including patients with endoscopic findings for peptic ulcer And chronic gastritis who referred to Tabriz Emam Reza hospital, were tested. H. pylori infection was confirmed in all patients by rapid urease test (RUT). DNA was extracted from positive urease test gastric samples. The presence of babA2 genotype was determined by polymerase chain reaction (PCR).

Results: Preliminary results are provided and the final results will be presented in Congress. Genomic DNAs were extracted from all strains. So far, 60 samples were examined by PCRs.

Conclusion: Discussion after obtaining the final results will be presented in Congress.

Keywords: Helicobacter pylori, peptic ulcer, Chronic gastritis, babA2 allele

P843: **Imipenem-resistant Pseudomonas aeruginosa strains carry vim-type metallo-beta-lactamases isolated from intensive care unit, Shahid Beheshti Hospital, North of Iran**

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Background and Aim:: Pseudomonas aeruginosa is the causing agent of many hospital infections and metallo-beta-lactamases (MBL) are being reported with increasing frequency. Objectives: The aim of this study was to determine the frequency of MBL among imipenem-resistant P.aeruginosa isolates and to compare methods of phenotypic and molecular detection.

Methods: During2011 to 2012, 50 P.aeruginosa samples were isolated intensive care units and tested for MBL production using phenotypic methods. Minimal Inhibitory concentrations (MIC) (µg/ml) were determined with commercial micro dilution panels. Pulsed Field Gel Electrophoresis (PFGE) was performed among MBL producers.

Results: Among 50 clinical isolates used in this study18 (%36) were found to be resistant to imipenem. Productions of MBL were detected in 15(30%) isolates with phenotypic method.PCR assay showed that 9(18%) isolates carried aVIM-1 gene. MBL- producing strains were shown 100% resistance to cefepime, ceftazidime, ceftriaxone, cefotaxime and imipenem. Amikacin and ofloxacin appeared to be the most active antimicrobial agent.

Conclusion: Since VIM metallo-beta-lactamases producing P. aeruginosa strains can cause serious infections that are difficult to treat, therefore, require rapid identification and the timely implementation of infection control measures in combination with systematic surveillance to monitor its potential clonal spread.

Keywords: Pseudomonas aeruginosa, metallo-beta-lactamases, Imipenem-resistant,

P844: **The Frequency of Epstein - Barr Virus in pediatric Hodgkin's Lymphoma in Iran**

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Background and Aim: Hodgkin's Lymphoma (HL), formerly called Hodgkin's disease, is a malignant tumor of the lymphatic system. The pathogenesis of Hodgkin's disease has been linked to Epstein-Barr virus (EBV). Pediatric Hodgkin Lymphoma occurring in developing regions is different from HL in industrialized countries due to the higher frequency of association with Epstein-Barr virus infection. In this study, we investigated the frequency of EBV in pediatric HL in Iran.

Methods: In this study, 16 Hodgkin's lymphoma tissue samples collected from country hospitals. Samples were selected from formalin-fixed paraffin embedded blocks and tested by in situ hybridization stains for EBV-encoded small RNA (EBERs) transcripts. Data analyzed by SPSS16 statistical software, Fisher's exact test.

Results: We studied formalin-fixed paraffin-embedded tissue from 16 pediatric cases with HL. The presence of EBV was assessed by Epstein-Barr encoded RNA in situ hybridization. Of the 16 cases, there were 12 boys and 4 girls, with a male to female ratio of 3: 1. EBV infection was identified in 75% of cases. EBER-positive HL cases included 9 boy samples (75%) and 3 girl samples (75%). Fisher's exact test showed statistically no significant difference between sex (p-value: 1.000). Epstein-Barr virus was present in different subtypes of Hodgkin's lymphoma. 4 of 5 (80%) with Mixed Cellularity (MC), 6 of the 9 (67%) with Nodular Sclerosis (NS) and 2 of 2 (100%) with Lymphocyte Predominance (LP) subtypes were positive. There is not significant different between subtypes (p-value: 0.826). The presence of Epstein-Barr virus in the age groups of 4-7 and 8-12 years were 71.5% (5 of 7) and 77.8% (7 of 9), respectively. There is not significant different between age group(p-value: 1.000).

Conclusion: Our results suggest that the frequency of EBV in pediatric HL in Iran is similar to other developing countries, also we found a striking association with expression of EBV- encoded small RNA (EBERs) transcripts in malignant cells in pediatric cases.

Keywords: Epstein-Barr Virus, Hodgkin's lymphoma, in situ hybridization

P845: **Potential activity of the Artemisia deserti flowers extracts against pathogenic bacteria**

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Background and Aim: Increased bacterial resistance to antibacterial agents is one of the most common problems in medicine. Plants used for traditional medicine contain substances that can be used to treat infectious diseases. Thus, the aim of this project was to determine antibacterial activity of methanol and chloroform extracts of Artemisia deserti flowers against selected pathogenic bacteria.

Methods: Artemisia deserti was collected from around area of Isfahan country (Golpaygan heights), Iran, in September 2012. The plant materials were dried under shade and ground into fine powder using electric blender. The flowers methanol and chloroform extracts were extracted successively with 1 L of methanol and chloroform, using a soxhlet apparatus for 4 h, separately. The extracts were dissolved in dimethylsulfoxide(DMSO) 20% at the concentrations of 30, 60,125, 250 and 500 mg/ml. Antibacterial activities were examined by micro dilution and agar well diffusion methods against Staphylococcus aureus, Enterococcus faecalis, Escherichia coli and Pseudomonas aeruginosa. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) was carried out by micro dilution method.

Results: Methanol extracts exhibited inhibitory effects against S. aureus, E.faecalis in range of 30 to 125 mg/ml. They did not have any activity against E.coli and P.aeruginosa. Thus, there are significant antibacterial effects between effective bacteria and control sample ($P<0.001$). The inhibitory effect of the extract was compared with standard antibacterial, Gentamicin and Vancomycin. Chloroform extract did not effective against tested bacteria.

Conclusion: Methanol extract of Artemisia deserti flowers inhibited growth of pathogenic gram positive bacteria. The results of this study indicate support the idea that medicinal plants can be promising sources of potential antimicrobial agents. These results form a good basis for selection of the plant for further phytochemical and pharmacological investigation.

Keywords: Artemisia deserti, antibacterial activity, extract, flower.

P846: **Viral gastroenteritis in Iran**

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Background and Aim: Background: Different types of viruses are the leading cause of acute diarrhea among infants and young children worldwide. Epidemiological surveillance of viral agents is critical for the development of effective preventive measures, including vaccines. This study aimed to determine the prevalence of the four major enteropathogenic viruses– rotavirus, norovirus, adenovirus and astrovirus– in children aged < 7 years old.

Methods: From October 2008 to September 2010, stool specimens of 375 children with acute gastroenteritis admitted to the Pediatrics Unit of 17 Shahrivar Hospital in Borazjan, Iran were investigated by using Enzyme Immunoassays (EIA).

Results: Rotavirus was detected in 91(24.27%) of the patients whereas the prevalence of norovirus, adenovirus and astrovirus was 12.53%, 5.1% and 2.4%, respectively. Averagely, 75.90% of children with viral diarrhea were younger than 2 years old. All studied viral gastroenteritis peaked in the autumn, except for adenovirus that was peaked in spring. The most common clinical symptoms included diarrhea (92.17%), vomiting (68.67%), abdominal cramp (60.84%) and moderate dehydration (57.23%).

Conclusion: Since nearly half of gastroenteritis cases (44.30%) were due to viral agents, testing for the viral antigens may guide the clinical approach to the patients with acute diarrhea particularly in children less than 2 years old and during the cold seasons.

Keywords: Gastroenteritis, Rotavirus, Adenovirus, Norovirus, Astrovirus

P847: **Immunological Investigation of Pseudomonas aeuginosa Detoxified Lipopolysaccharide as a Protective Vaccine in Mouse Model**

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Background and Aim: Therapy of Pseudomonas aeruginosa infections with antibiotics is difficult due to antibiotic resistance of the organism. Hence, the immunoprophylaxis and immunotherapy might be an effective method for treatment and control of P. aeruginosa infections. LPS-based vaccines can prevent human Pseudomonas infections. Therefore, LPS of this bacterium could be a candidate for new generation development of conjugate vaccines. In this research, protection properties of detoxified lipopolysaccharide (D-LPS) against P. aeuginosa infections were evaluated in mice.

Methods: LPS was extracted from P. aeruginosa strain PAO1 by hot phenol method with some modifications. LPS detoxified by NaOH. D-LPS was assayed by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) and by the rabbit thermal induction and the Limulus amebocyte lysate (LAL) test. After molecular evaluation, protective activities and safety of the D-LPS were determined in mice model. Fifteen female BALB/c mice were immunized intraperitoneally on days 0, 14 and 28 with 10 µg of D-LPS. Control group were injected only with normal saline. An enzyme-linked immunosorbent assay (ELISA) for measurement of antibody to LPS was performed.

Results: D-LPS was non-pyrogenic and did not produce any detectable abnormal toxicity in rabbits and mice. Endotoxin content was 0.125 EU/ml by LAL assay. The third immunization with D-LPS induced high levels of antibodies in mice. No specific antibodies were detected in mice in the negative control group.

Conclusion: The results showed that immunization with D-LPS produced significant antibodies in mice model and can be used as a profit and protective immunogenic in vaccine design against infections caused by P. aeruginosa.

Keywords: P. aeruginosa, LPS, Vaccine, Immunization

P848: **Immunogenicity Evaluation of Alginate and Detoxified Lipopolysaccharide of Pseudomonas aeuginosa as Vaccine Candidate Antigens in Mice Model**

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Background and Aim: Pseudomonas aeruginosa continues to be a leading cause of life-threatening nosocomial infections. Alginate and lipopolysaccharide (LPS) are important virulence factors in P. aeruginosa that isolated from infected persons. Immunity against these components confers protection against P. aeruginosa infections. The aim of this study was to evaluate the immunogenicity of alginate and detoxified LPS (D-LPS) from P. aeruginosa in mouse as vaccine candidate.

Methods: Alginate and LPS were purified from P. aeruginosa of strain PAO1. Alginate was isolated by repeated ethanol precipitation, dialysis, enzymatic digestion, and chromatography. Alginate was depolymerized by controlled heating in dilute acid. LPS was extracted by the hot phenol procedure and detoxified by NaOH. The level of pyrogenicity was determined by intravenous administration of graded doses of antigens to rabbits. 30 mice in two groups were immunized intraperitoneally on days 0, 14 and 28 with 10 µg of alginate and D-LPS. Then serum samples were collected and enzyme-linked immunosorbent assays (ELISA) for the quantitation of anti-alginate and anti-LPS antibodies were performed.

Results: Alginate and D-LPS were non-pyrogenic when administered to rabbits at a dose of 10 µg/kg of body weight. While D-LPS given in a range of doses was poorly immunogenic in mice, the alginate induced high levels in all types of total IgG, IgM, IgA, IgG1, IgG2a, IgG2b and IgG3 antibodies.

Conclusion: The results of this study showed that alginate is a highly immunogenic polysaccharide in versus D-LPS, thus alginate can be used as a vaccine candidate in other studies.

Keywords: P. aeruginosa, LPS, Alginate, Vaccine Candidate

P849: **Molecular epidemiology of the Staphylococcus aureus by Rep-PCR method in Sanandaj hospitals**

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Background and Aim: Staphylococcusaureus is a major pathogen within hospital and in the community. The aim of this study was the showing of genetic relationship in isolates and transmission in hospital.

Methods: Eighty eight S. aureus strains isolated from different clinical samples and were characterized by Repetitive extragenic palindromic (Rep–PCR) technique.

Results: . The received results and the similarity between the strains were determined on the basis of the Jaccard similarity coefficient in the SAHN program of the NTSYS-pc software. The Rep–PCR profile allowed the typing of the 88 isolates into 7 main clusters.

Conclusion: In conclusion, our results showed more diversity in S.aureus isolates that indicates the low rate of hospital infection in Sanandaj hospitals and the results of the share pattern of especially among ICU,Pediateric and Internal wards indicate that rate of nosocomial infection are high in wards.

Keywords: S. aureus,genetic diversity,nosocomial infection ,DNA fingerprinting

P850: **Prevalence of dfr, int and sul genes among Klebsiella pneumoniae isolated from hospitals of Ilam and Milad hospital of Tehran**

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Background and Aim: Extensive use of antimicrobial agents such as cotrimoxazole has been associated with raising of antimicrobial resistance. Current study is focused on assessing the prevalence of cotrimoxazole resistance in klebsiella pneumonae and the frequency of related genes.

Methods: 155 isolates of klebsiella pneumonae were collected during Mar.2007 to Apr.2012 from Ilam hospitals and Milad hospital of Tehran. Antibiotic susceptibility test done to screening resistance isolates according to Kirby-Bauer method .sul1, sul2, sul3, dfrA1, dfrA5, and Int1 genes were detected by PCR.

Results: Among 155 species, forty isolates (26%) were resistance to cotrimoxazole. frequency of sul1 gene was 32 isolates (80%) and 24 isolates of dfrA1(60%), none isolates of dfrA5 (0%), 28 isolates of int (70%), 25 isolates of sul2 (62.5%), and no isolates of sul3 (0%) has been detected. 17 (42.5%) isolates have sul1 and sul2 simultaneously, and 18 (45%) isolates have int1 and dfrA1. 11 isolates have sul1,sul2,int1 and dfrA1 genes concurrence by 27.5% frequency.

Conclusion: Our study shown resistance to Cotromoxazole in klebsiella isolated from Ilam hospitals and Milad hospital of Tehran is moderate and sul genes have the highest frequency in resistance isolates.

Keywords: Cotrimoxazole, sul1 , Resistance gene, Klebsiella, Tehran, Ilam.

P851: **MicroRNAs dysregulation in response to Helicobacter pylori infection in gastric diseases**

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Background and Aim: Helicobacter pylori (H. pylori), a gram-negative microaerophilic bacterium, infects the gastric mucosa of about half of the world population. H. pylori is a major human pathogen that plays a considerable role in gastric diseases such as peptic ulcer, gastritis, mucosa-associated lymphoid tissue (MALT) lymphoma and gastric cancer. Gastric cancer is the second leading cause of cancer death worldwide and the Union for International Cancer Control has documented H. pylori as a group I carcinogen. H. pylori eradication has been shown to have a prophylactic effect on gastric cancer. H. pylori infection induces huge array of responses at the gastric epithelial cells and the immune system. Among the mediators induced in response to the infection, microRNAs (miRNAs) have the potential to play a major impact on the outcome of the bacteria-host interaction.

Methods: miRNAs are small non-coding RNAs of 18–24 nucleotides that are currently considered as crucial post-transcriptional regulators of gene expression. These miRNAs are found to regulate genes involved in many biological processes, including development, differentiation, apoptosis, cell cycle progression, angiogenesis, proliferation and signal transduction pathways. They are also implicated in the regulation of immune responses and host defense against pathogens.

Results: Alterations in microRNA expression have been linked to the pathogenesis of many malignancies and reveal their functions as either oncogenes or tumour suppressors. So miRNAs can be used as biomarkers for early diagnosis of different tumours and the regulation of their expression may be a new strategy in cancer treatment. Bacteria have evolved strategies that suppress miRNA functions, resulting in a sustainable infection.

Conclusion: Recent studies have shown some miRNAs could be altered after H. pylori infection and involve in gastric carcinogenesis. In this review, miRNA changes in response to H. pylori infection and potential etiologic roles they play in H. pylori-mediated gastric carcinogenesis are summarized.

Keywords: Helicobacter pylori, microRNA, gastritis, gastric cancer

P852: **The effect of chronic stress on the efficiency of influenza gene vaccines to induce immune responses against influenza virus in mouse model**

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Background and Aim: Influenza is a highly contagious and acute respiratory disease caused by infection of the host respiratory tract mucosa by influenza virus. The use of DNA-vaccines that expressed conserved genes such as NP represents a novel approach to vaccination against influenza. In this study, the effect of chronic stress on the efficiency of this type of vaccine has been evaluated in a mouse model.

Methods: Male BALB/c mice 6 –7 weeks of age were randomly allocated to four groups: a) vaccinated with stress b) vaccinated without stress c) PBS injected and d) Influenza/PR8 injected group. The stressed groups were stressed 6 hours a day for 2 weeks using restraint stress procedure, and then they were vaccinated with NP-DNA vaccine. (B, c and d groups were injected with NP-DNA vaccine, PBS and influenza/PR8 respectively). After vaccination stressed mice were stressed for 2 weeks, again. Then spleens of all mice were removed and CTL response was evaluated with flowcytometry and lymphocyte proliferation rate was measured with XTT cell proliferation kit.

Results: The results indicate a significant reduction in the CTL response of stressed mice in comparison with mice without stress. Also the lymphocyte proliferation rates in stressed mice were less than mice without stress.

Conclusion: Restraint stress modulated the immune response to DNA vaccination against influenza virus in mice. Chronic stressed may sufficiently down-regulate immune response in mice model so that to delay and/or inhibit the synthesis of adequate levels of T-cell response sufficient to provide adequate protection to an influenza infectious agent.

Keywords: influenza, DNA vaccine, stress

P853: **Detection of E7 region of HPV 16 DNA gene in cervical dysplasia by PCR methods**

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Background and Aim: Background Cervical cancer is a common neoplasm of the female genital tract and continues to be the most deadly female cancer in developing countries. In order to progress a cancer from dysplasia to invasive carcinoma, a series of cellular changes should occur. High-risk Human papillomavirus (HPV), in particularly HPV 16 are associated with cervical carcinoma that due to the ability of the viral oncoproteins, E6 and E7, to abrogate the cell cycle. It is believed that HPV can increase the rate of cancer progression when associating with other risk factors such as smoking, taking contraceptive drugs and etc. The present study was conducted to detect the prevalence of HPV16 infection in Iranian women.

Methods: Methods Formalin fixed paraffin-embedded samples from patients with cervical cancer were processed (archival pathologic specimen of Mirza, Emam Khomeini and pars Hospitals) that confirmed by pathologists. We purified DNA and utilized PCR method for detection E7 region of HPV 16 DNA of 69 patients with cervical cancer.

Results: Results Among total patients, 51 % were positive for HPV16 that show importance of HPV16 in prevalence of cervical carcinoma. The highest number of patients belonged to age-group 41-50 , OCP consumer and women who married under 18 years old.

Conclusion: Conclusion In our experience, the PCR technique is a robust, simple and sensitive way of type specific detection of HPV16E7 genes in Paraffin embedded tissue that makes this technique applicable to routine practices of HPV detection. HPV 16 is one of the major genotypes that associated whit cervical carcinoma in Iran.

Keywords: E7 .HPV 16. genotype.PCR.Dysplasia

P854: **Comparison of the Acinetobacter baumannii resistance pattern on Nanosilver with Amikacin and Gentamycin antibiotics**

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Background and Aim: Acinetobacter baumannii is recognized to be among the most difficult antimicrobial-resistant gram-negative bacilli to control and treat. The Aminoglycosides are still the most commonly used antibiotics to Gram-negative bacterial strains with advanced patterns of antimicrobial resistance. In this survey, we examined the efficacy of Nanosilver material with strong disinfectant properties against A.baumannii and compared with Amikacin and Gentamycin antibiotics.

Methods: The 60 A.baumannii strains bacteria were isolated from clinical specimens of university hospitals in Tehran providence of Iran. The susceptibility test against Amikacin and Gentamycin antibiotics was investigated by Disk diffusion methods. Colloidal Nanosilver with about 6-34 nm particle size was prepared by chemical methods. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) effects of Nanosilver on A.baumannii were assessed at serially diluted Nanosilver and compared with used antibiotics according to CLSI methods.

Results: The results showed the resistance of A.baumannii was 29.32% against Gentamycin and 12.07% against Amikacin .The MIC and MBC Mean of Nanosilver dilutions were determined 27.34 ppm and 54.68 ppm, respectively all isolates (100%) were susceptible to more than MIC of Nanosilver solutions.

Conclusion: It is well known that this organism develops rapid resistance to various groups of antimicrobials including aminoglycosides but it is still choice on multidrug resistance gram negative bacteria although their side effects of renal and auditory system were recognized. We conclude that nanosilver is strong bactericidal agent for the control of the A.baumannii bacteria , so it can be useful to prevent variety of nosocomial infections.

Keywords: Acinetobacter baumannii, Amikacin, Gentamycin, MIC, MBC, Nanosilver

P855: **To Consider Impression OF The Carvacrol On The Salmonella Enterica PTCC 1709 And Staphylococcus Aureus ATCC 6538**

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Background and Aim: In the recent years, appearing bacteria resistance to antibiotics were increased . one of the ways for comparing with this problem is acquaintance new compounds with natural sources . essential oil of Satureja contains about 90% carvacrol in the during and before flowering stage . according to antibacterial effects of phenolic compounds such as, thymol and carvacrol, antibacterial effects of Satureja Khuaistanica were investigated on S. enterica , S. aureus bacteria. The major aim of this investigation was comparison effect carvacrol existing in the of essential oil Satureja Khouaistanica that available of the Satureja leaves plants with 15 antibiotics on the Staphylococcus Aureus and Salmonella enterica that use from cure those in vitro .

Methods: To inquiry the antibacterial properties of carvacrol existing in the essential oil of Satureja Khouaistanica , has been used Disk Diffusion method and dilution in the micro plate .then diameter of inhibition zone was determined for S. enterica , S. aureus bacteria respectively 16 ،30 mm .Result of the minimum inhibitory concentration (MIC)showed for S. enterica , S. aureus bacteria respectively 0/78 ، 0/19µg/ml while their minimum bactericidal concentration(MBC) indicated for S. enterica , S. aureus bacteria respectively 1/56،0/39µg/ml. the diameter of inhibition zone for 15 antibiotics used in the clinical cure ranged between 2 to 14mm for S. enterica bacteria and S. aureus bacteria.

Results: According to the results, considerably carvacrol existing in the essential oil of Satureja Khuaistanica, indicated that antibacterial activity on mentioned bacteria.

Conclusion: The results showed that, these carvacrol existing in the essential oil of Satureja Khouaistanica possess antimicrobial effects, considerably, thus, it seems can be suitable alternative to use of synthetic antibiotics that due to resistance in bacteria.

Keywords: carvacrol ,essential oil of Satureja Khouaistanica , S. enterica , S. aureus

P856: **Prevalence of bacteria causing meningitis in patients admitted to the Medical Centers of Shahid Beheshti University**

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Background and Aim:: Bacterial meningitis is a serious infectious disease, that lack of early diagnosis can be associated with mortality. The aim of this study is identify common bacteria causing meningitis in hospitalized patients in Shahid Beheshti university medical centers.

Methods: In this study, 55 children with meningitis patients admitted to hospitals since April 2010 to May 2013 were analyzed in terms of the "Blood and CSF cultures "and "age, sex and season distribution".

Results: Results: Of the 55 studied Patients, 29 (52.73%) had aseptic meningitis and 26 cases (47.27%) had bacterial meningitis. Classification of these patients in two types of meningitis was made by CSF. Minimum and maximum age of patients were one month and 13 year, respectively. The mean age of patients was 24 months. Common genus with 32 (58.2%) were male. The peak of meningitis prevalence (36.4 %) was winter. Blood and CSF cultures were positive in 7% of cases. The CSF cultures in 16.4% and blood cultures in 23.6% were positive, in itself. The most common isolated bacteria were Staphylococcus epidermidis (4.4%), Streptococcus pneumonia(11.4%), Staphylococcus aureus (6.5%), Pseudomonas aeruginosa (10%), Neisseria meningitidis and Escherichia coli were 9% and 6%, respectively. The most common treatment for meningitis was included Ceftriaxone and Vancomycin. The mortality rate in this study was three.

Conclusion: Based on finding of this study, the main factors causing bacterial meningitis were "Streptococcus pneumoniae" and "Neisseria meningitidis" and " Pseudomonas aeruginosa".

Keywords: meningitis, bacteria.

P857: **Investigation of the effects of the Yersinia protein yopM on the proteome of HEK293 cells**

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Background and Aim: Yersinia are known to code the effector protein yopM which interacts with the human host cell kinases RSK and PNK. However, it is not yet known which host cell proteins will get affected upon the interaction of yopM with RSK and RNK. For identifying these proteins a differential proteomic approach applying two-dimensional proteomis was chosen. HEK293 cells were transfected either with yopM or with the PCMX-vector as control. After 48 hours the cells were starved on a serum-free medium and washed several times with PBS. After adding protease and phosphatase inhibitors the cells were lysed and subjected to two-dimensional electrophoresis. By this technique intact proteins are separated with high resolution. With a 2DE gel more than 1000 proteins were resolved. In the differential proteomic approach the 2DE protein patterns of the control and the perturbated sample were compared. Those protein spots were picked, which differ in their intensity, the proteins digested with trypsin and the desalted tryptic peptides analysed by mass spectrometry, followed by a data base search of the mass spectrometric data versus a protein data base with a search engine. The identified proteins are giving more insight into the action of yopM in the host cells of yersinia.

Methods & Results & Conclusion: Yersinia are known to code the effector protein yopM which interacts with the human host cell kinases RSK and PNK. However, it is not yet known which host cell proteins will get affected upon the interaction of yopM with RSK and RNK. For identifying these proteins a differential proteomic approach applying two-dimensional proteomis was chosen. HEK293 cells were transfected either with yopM or with the PCMX-vector as control. After 48 hours the cells were starved on a serum-free medium and washed several times with PBS. After adding protease and phosphatase inhibitors the cells were lysed and subjected to two-dimensional electrophoresis. By this technique intact proteins are separated with high resolution. With a 2DE gel more than 1000 proteins were resolved. In the differential proteomic approach the 2DE protein patterns of the control and the perturbated sample were compared. Those protein spots were picked, which differ in their intensity, the proteins digested with trypsin and the desalted tryptic peptides analysed by mass spectrometry, followed by a data base search of the mass spectrometric data versus a protein data base with a search engine. The identified proteins are giving more insight into the action of yopM in the host cells of yersinia.

Keywords: yopM, 2DE, mass spectrometry

P858: **Evaluation of Induced Immune Responses Against Staphylococcus.aureus Capsular Type 5 by Its Conjougate to Diphtheria Toxoid**

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Background and Aim: Staphylococcus aureus is the most common nosocomial Pathogen and is Responsible for approximately one-third of hospital acquired bacteraemias.The discovery of S.aureus capsular polysaccharedes from clinical isolates and their importance to pathogenicity via anti-phagocytic Activity opened a nem window of opportunity for development of vaccines And immunotherapy against this pathogen .Purpose of this study was to evaluate immunogenicity of Capsular polysaccharide of S.aureus with Diphteria toxoid as a conjugate vaccine in mice and measuring antibodies titers in conjugate form with Diphtheria toxoid.

Methods: The capsule was isolated from bacterial extract by cold ethanol and enzyme digestions,Then purified by ion-exchange Chromatography.The purtified capsule was coupled to DT with ADH as a spacer and EDAC as a linker. The reaction mixture was passed through a sepharoseCL-2Bcolumn.pyrogenicity test shown that resulting conjugate was non-toxic and non-pyrogenic. then four group of female BALBc mice was selected. vaccination was performed by three doses with two week interval. Then serum samples was collected and antibody responses against CPS5-DT was measured by indirect ELISA method for total Antibodeis.

Results: Two weeks after first dose there was no significance different between antibodies titers in groups that were immunizided by CPS5 and CPS5-DT.But after second and third doses CPS5-DT showed significance increasing in all types of antibodies titers.The results of anti CPS inductions for total antibodies were observed CPS5-DT more of CPS5 more of DT

Conclusion: These result indicated that CPS from Type5 Staphylococcus aureus increases antibodies titers in conjugated form with Diphtheria toxoid and can be an appropriate effective conjugate for vaccines

Keywords: conjugate-ELISA-Chromatography- Capsullar polysaccharied -Staphylococcus aureus

P859: **Biosynthesis of silver nanoparticles using pseudomonas sp1 and its antimicrobial activity against Candida. albicans**

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Background and Aim: Recent advancements in science specially nanotechnology and human increasing requirements to produce nanoparticles by means of chemical methods, is the perpose of nowadays researchers trying to produce nanoparticles by biological methods. Since the silver nanoparticles high consumption are growing in different industries and medicine. For screening of mine soil, in Golgohar mine, located in Kerman, sirjan, to find silver nanoparticles bacteria production and the study of nanosilvers activity against Candida. albicans have drawn.

Methods: For this matter, samples of soil were collected from diffrent parts of mine and the samples in sterile condition sent to laboratory. Isolatin and purification of mesophillic aerobic bacteria was conducted. All isolates were cultivated in a separate nutrient broth without sodium choloride and incubated in 30 o C in 48h and 120rpm. After centrifuge, the supernatant was mixed with silver nitrate in 0.001 mM in equal proportion. After 72h, observation of brown color and the most absorbtion in 440 ƛ, was assessed.

Results: Among the isolates, The starin that recognized to pseudomonas sp1 has the ability to produce nanoparticles and antimicrobial test against Candida. albicans which was prepared from PTCC No. 5027 with well-diffusion method has been effective.

Conclusion: According to the results, Silver nanoparticles which produced by pseudomonas sp1 can prevente the development of Candida. albicans PTCC 5027. It is recommended to generate nanoparticles in a high scale to disinfect surfaces.

Keywords: Screening, mine soil, Silver nanoparticles ,Candida. albicans

P860: **Antifungal activity of Hypericum perforatum against human pathogenic dermatophytes**

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Background and Aim: Hypericum perforatum is one of the important medicinal plants with high antifungal properties. The plant grows in a wide range of areas of Iran including Tehran and Guilan provinces.

Methods: Flower extract of H. perforatum was prepared using methanol as solvent in serial two-fold concentrations of 2000-15.6 µ/ml. The antifungal activity of extract against four dermatophyte species was studied by the disk diffusion assay. The fungi were cultured on Muller Hinton agar medium and impregnated disks were applied on the culture plates. The plates were incubated at 35 ºC for 5-7 days. Diameters of inhibition zones (mm) were measured.

Results: The results showed that methanolic extracts of H. perforatum flowers, roots and stems had fungistatic and fungicidal effects on the dermatophytes tested with inhibition zones of 0.0-6.0 mm.

Conclusion: Our results showed that H. perforatum methanolic extracts had potent antifungal properties against pathogenic dermatophytes. This antifungal activity can be attributed to hypericin as the principal plant component.

Keywords: Hypericum perforatum; Extract; Pathogenic dermatophyt; Antifungal activity

P861: **Comparison Lactobacillus casei and L. fermentum CFCS as an inhibitory factor of Shigella sonnei and Shigella flexneri in MDR pattern**

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Background and Aim: Shigellosis remains an important public health problem by S. sonnei and S. flexneri in US, Europe and in Asian, gringo in developing countries. Shigella sp. is one of the most antimicrobial-resistant bacteria and important causes of gastroenteritis. Preparing the prevention and treatment protocols with natural patterns in this regard seems to be necessary. Recent reports have documented the role of Lactobacillus in the prevention and treatment of some infections. Lactobacillus strains have commensally in the human body. Its beneficial effect may be associated to its ability for inhibiting the growth of pathogens, apparently by the secretion of antibacterial substances including lactic acid, hydrogen peroxide and etc. This study evaluates the protective effect of Lactobacillus casei and L.fermentum cell-free culture supernatants (CFCS) against multiple drug resistance (MDR) clinical samples of Shigella sonnei and Shigella flexneri in vitro. Also, this study compared the antimicrobial effects of two lactobacillus strains on MDR Shigella spp. Furthermore, we estimate the tolerance properties to confirm the L. casei supernatant could be potentially use as an inhibitory proliferation factor.

Methods: S. sonnei and S .flexneri was identified by common microbiological and serological methods. Antibiograms with 18 antibiotics were tested for 34 positive cultures by disc diffusion method. The Samples showed considerable resistance to antibiotics. Antimicrobial effects of CFCS were tested against S. sonnei and S. flexneri by agar-well assay and broth micro dilution methods. In addition, the antimicrobial activity remained active treatment after adjust pH 7, adding Proteinase K and heating for L. casei and L.fermentum.

Results: The results implicate that L. casei and L.fermentum strongly inhibits the development of pathogen samples. In contrast, via the disc diffusion method 4 out of 18 antibiograms have shown complete resistance against the pathogen samples. In addition, the natures of antimicrobial properties have been tested in different conditions such as various pH, temperature and presence of proteinase K. The MIC50 (minimum inhibitory concentration) and MIC90 of CFCS of L. casei and were determined, for S. sonnei were 2.25 and 10.5, for S .flexneri were 5.25 and 5.25 respectively. Similarity for L.fermentum respectively were 5.25, 5.25, 10.5 and 21 (µl/ml). The results have shown a significant resistance pattern by these four antibiotics in this case.

Conclusion: The data indicates that. L. casei and L.fermentum highly resistant against to antibiotics, heat, Proteinase K and so many activities against MDR Shigella pathogenic strains . L. casei is the best probiotics candidate. Also L.casei had better effect than L.fermentum.

Keywords: Lactobacillus casei, L. fermentum, Shigella sonnei, Shigella flexneri

P862: **Prevalence of Beijing genotype of Mycobacterium Tuberculosis in tuberculosis patients in Tehran 2012**

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Background and Aim: Mycobacterium tuberculosis strains of the Beijing genotype were 1st identified in Beijing China and neighboring states. This genotype has been associated by numerous epidemics. According to minute data in Iran, the aim of this study was to evaluate the frequency of this genotype in Tehran.

Methods: A set of 32 strains obtained from patients living in Masih Daneshvari hospital, Tehran was used to evaluate the capability of the Beijing TaqMan® real-time PCR assay established in this investigation for discrimination of beijing and non-beijing genotype strains.

Results: From the total samples obtained was 9.37% of the sample belonged to the beijing genotype. The all patients were male and mean age 25 years old.

Conclusion: Results showed that multi-drug-resistance was more prevalent in bacteria isolated from Afghan TB patients residing in Iran. In addition, the spread of M. tuberculosis strains belonging to beijing family among Iranian patients has to be considered seriously. It is also important to undertake studies to identify which factors are the most significant to consider in tuberculosis control program.

Keywords: Mycobacterium tuberculosis, Beijing genotype, Tehran

P863: **Efficacy of microwave disinfection on dental stone casts**

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Background and Aim: Dental practice contains the use of instruments and multiuse items that should be sterilized or disinfected properly. The aim of the current study was to investigate the effect of microwave irradiation on dental stone cast disinfection in moist and dry condition.

Methods: In this in vitro study, 76 stone casts were prepared by a sterile method. The casts were contaminated by Pseudomonas aeruginosa(ATCC 9027), Staphylococcus aureu(ATCC 6538), Enterococcus faecalis (ATCC 29212)as well as Candida albicans(ATCC 10231). Half the samples were dried for two hours and the other half was studies while still moist. The samples were irradiated by a household microwave at 600 W for 3, 5 and 7 minutes. The microorganisms on the samples were extracted by immersion in tryptic soy broth and .001 ml of that were cultured in nutrient agar media, incubatedovernight and counted and recorded as colony forming unit per milliliter (CFU/mL).

Results: The findings showed that microorganisms were reduced to 4.87 logarithm of CFU/mL value on dental cast within seven minutes in comparison with positive control. Although microbial count reduction was observed as a result of exposure time increase, comparison between moist and dried samples showed no significant difference.

Conclusion: Seven-minute microwave irradiation at 600 W can effectively reduce the microbial load of dental stone casts. Wetting of the casts does not seem to alter the efficacy of irradiation.

Keywords: Microwave, Dental stone casts, Disinfection

P864: **The presence of resistance genes to aminoglycosides in Pseudomonas aeruginosa strains isolated from different parts of the shahid Rajai Hospital using PCR techniques**

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Background and Aim: Pseudomonas aeruginosa is a nosocomial important pathogen. Treatment of infections caused by this bacterium to cause high resistance to most antibiotics is difficult. Pseudomonas aeruginosa can acquired resistance to antimicrobial agents such as aminoglycosides, beta lactamase, fluoroquinolone. Aminoglycosides are a important combination of antipseudomonal chemotherapy. The study aimed to the presence of resistance genes to aminoglycosides in Pseudomonas aeruginosa strains isolated from different parts of shahid Rajai Hospital was investigated using PCR techniques.

Methods: In this study, 35 strains of Pseudomonas aeroginosa were collected from different wards of the Shahid Rejaei hospital of Tonekabon within 2011-2012. In order to determine the resistance of strains, antibiogram test was carried out by the method of the disk diffusion. In order to the presence of the studied genes, the Specific primers were used and the PCR technique was applied to amplification of the resistance genes to aminoglycosides.

Results: All strains showed different levels of resistant to antibiotics including, 42.8% to Toberamycin, , 20% to Cephtazidim and 2.8% to Imipenem, 42/9% to Toberamysin, 85/8% to Neomycin. All Pseudomonas aeroginosa isolates were sensitive 100% to Ciprofloxacin and Amicacin. Of total 35 studied strains, aadE gen was observed, in 33 (94/2%) strains, respectively. In the present study aadA, and aac(6'), aph (2 '') genes of the studied strains wasn't detected thus in this study between the mechanisms of aminoglycoside resistance genes, gene aadE was superior.

Conclusion: Pseudomonas aeruginosa resistant to aminoglycosides has been found in many hospitals. Studies have shown that the presence of resistant organisms is related to the amount of aminoglycosides used at a specific section.With regard to the high percentage of the resistance of isolated Pseudomonas aeroginosa to the aminoglycoside antibiotics, the accurate performance of the antibiogram tests before the prescription of the antibiotic in the treatment of the infections resulted from these bacteria is an unavoidable necessity.

Keywords: Pseudomonas aeroginosa, antibiotics Resistance, Aminoglycosides

P865: **Purification of two antigens extracted from Bordetella pertussis as the potential candidates for producing of an acellular vaccine in Iran**

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Background and Aim: Acellular pertussis vaccine mainly composed of two extracellular proteins of Bordetella Pertussis including of pertussis toxin (PT) and filamentous hemagglutinin (FHA) .The aim of this study was to purify these two antigens from submerged culture of B. Pertuusis using affinity and adsorbtion chromatography in order to produce an acellular vaccine in Iran.

Methods: For this purpose B. Pertussis strain RIVM - 134 was grown in a 45-litre fermentor using a modified Stainer–Scholte medium supplemented with dimethyl (2,6-o-) β -cyclodextrin . The two antigens (PT and FHA) were extracted from the supernatant of the culture after centrifugation and was concentrated 20 times and then submitted to chromatography for purifing of the above two antigens.

Results: Pertussis toxin from one of the fractions in Fetuin - Sepharos 4B column and FHA from a certain fractions in Hydroxylapetite column were extracted, dialysed and protein concentration of 1200, 950 ?g/ml were estimated for PT and FHA respectively.The entity of both antigens were confirmed by SDS-PAGE and immunoblotting techniques.

Conclusion: The appropriate selected strain and the purification method are two main factors that contribute to the success in PT production in high yeilds. In current study strain 134 was selected for producing of PT on the basis of its capacity to produce toxin in the pertussis vaccine production procedure. The srain showed considerable growth in Stainer-Scholte medium and under the conditions employed good yeilds of PT can be obtained from this strain. Furthermore, the results showed that the purifing of PT and FHA are at their best when Fetuin-sepharose 4B and hydroxylapatite columns have been used.

Keywords: Pertussis, Acellular vaccine, PT, FHA antigen, Purification

P866: **Comparision of antibacterial effects of various samples of honey**

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Background and Aim: In this study , antibacterial effects of samples honey of different areas of Iran for prevention and treatment of infection subsequent burn ,were compared

Methods: After extraction of comb honey,PH,refractive index and water perecentage of all of the samples of honey were measured.Than antibacterial effects of these samples as pure and 80and 40 percent weight volume of samples were studied.To do this study paper disk method was used , becouse of the advantages of this method in comparison to other diffusion methodes.After preparation of inoculum of Staph aureus,Escherishia coli, Pseudomonase, Klebsiella,Entrobacter,and comparison with McFariand solution NO: 0.5.and inoculated on the surface of Mueller Hinton agar.Than paper disk soaked weltering in pure and 80 and 40 percentof honey were placed on the inoculated medium after incubation for period 16-18 hours

Results: After extraction of comb honey,PH,refractive index and water perecentage of all of the samples of honey were measured.Than antibacterial effects of these samples as pure and 80and 40 percent weight volume of samples were studied.To do this study paper disk method was used , becouse of the advantages of this method in comparison to other diffusion methodes.After preparation of inoculum of Staph aureus,Escherishia coli, Pseudomonase, Klebsiella,Entrobacter,and comparison with McFariand solution NO: 0.5.and inoculated on the surface of Mueller Hinton agar.Than paper disk soaked weltering in pure and 80 and 40 percentof honey were placed on the inoculated medium after incubation for period 16-18 hour

Conclusion: The diffrence in antibacterial activities among various samples of honey may be due to chemical composition of nectar of flowers of different areas, climatic conditions and other factors.Also antibacterial effect of a given sample of honey was different,on various bacterial species, and furthermore the concenteration of honey in which the most potent antibacterial activity was observed differed for each bacterial specias.

Keywords: antibacterial , honey

P867: **Diagnosis of Staphylococcus aureus sinusitis with Loop mediated isothermal amplification (LAMP)**

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Background and Aim: Sinusitis is the medical term for inflammation of the sinuses. It's usually caused by infection. LAMP is a powerful innovative gene amplification technique emerging as a simple rapid diagnostic tool for early detection and identification of microbial diseases. One of the stubborn agents in sinusitis diseases is staphylococcus aureus. Our purpose is rapid detection of staphylococcus aureus sinusitis by novel molecular technique.

Methods: The specimens (55) were provided from the biopsy of maxillary and frontal sinuses of hospital patients. Genomic bacterial DNA was extracted by DNG-plus kit and was amplified employing sequence-specific target namely the nuclease gene. Primer design carried out by Primer explorer ver, 4 in Eiken site for nuc gene. Sensitivity tests have been done for LAMP test and then optimized for the samples. At the end, LAMP products have been examined by percipated Mg2P2O7.

Results: LAMP test was optimized with the Bst large fragment DNA polymerase in 66°C for 60 min. Sensitivity test contained about ten S.aureus particles and no result was obtained by any of tested DNA during specification test. The results showed that regarding S.aureus DNA existence 19 of 55 samples were positive in the sinusitis samples.

Conclusion: The LAMP assay allows a one-step identification of gene of interest without any specialized equipment and also the technique might be an appropriate alternative diagnostic method for detecting nuc gene of S. aureus in sinusitis samples.

Keywords: LAMP ,Staphylococcus aureus, diagnosis, sinusitis

P868: **Antimicrobial activity of polypeptide purified from Iranian scorpion Hemiscorpius lepturus**

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Background and Aim: Nowadays antibiotic-resistant bacteria especially Pseudomonas aeruginosa, as an opportunistic pathogen, seriously threaten human health. Antimicrobial peptides are small natural molecules exist in the widespread groups of organism which have an effective role against infectious diseases caused by bacteria and other microbes. Scorpions are one of the most ancient animals on the earth which are considered as appropriate source to obtain antimicrobial peptides. Hemiscorpius lepturus (H. lepturus) is one of the most dangerous scorpion in southwest of Iran which has a highly cytotoxic and neurotoxic venom. In this study antimicrobial effects of FPLC-purified polypeptides were investigated.

Methods: Venom was obtained by electric stimulation, dissolved in the distilled water, and then centrifuged at 14,000 rpm for 15 min. BCA method was used to determine protein concentration at 562 nm. Supernatant was applied to a Sephadex G-50-superfine column (1.6cm x 110 cm) equilibrated with buffer ammonium acetate. Electrophoretic analysis of venoms was performed on 18% polyacrylamide gel. The obtained FPLC fractions were tested for their antibacterial activity against Pseudomonas aeruginosa ATCC 27853. Inhibitory activity was determined by the disk diffusion method.

Results: Of the 7 protein fractions obtained from Sephadex-G50, only two fractions had peptides less than 10 kDa. It was observed that H. lepturus venom showed antibacterial effect against P. aeruginosa on Muller Hinton agar and the purified fraction gave a clear zone diameter.

Conclusion: Scorpion species often use their venom to disinfect themselves from saprophytic organisms including bacteria and fungi. This potential shows that venom of scorpions could contain some sort of antibiotics. Using of natural compounds such as antimicrobial peptides derived from various organisms could be an effective solution to deal with multidrug-resistant pathogens. The results demonstrated that a peptide/protein fraction is responsible for antibacterial activity in the scorpion venom. This study is pending to purify the effective fraction using Reverse Phase HPLC and re-examine the antibacterial activity against more bacterial species.

Keywords: Antimicrobial peptide, Hemiscorpius lepturus, Pseudomonas aeruginosa, FPLC

P869: **Correlation of extended-spectrum β -lactamase phenotype with blaSHV, blaTEM and blaCTX-M gene carriage in urinary isolates of Klebsiella pnemoniae in Tehran**

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Background and Aim: Klebsiella pneumoniae is an opportunistic pathogen that causes a significant proportion of community and hospital acquired infections Among the antibiotic classes, β-lactams are often used for treatment of these infections. However, bacterial resistance has developed mostly due to the production of a variety of β-lactamases, especially the extended-spectrum β-lactamases (ESBL).

Methods: We examined ESBL production in urinary isolates of K. pneumoniae in relation to the presence of blaSHV, blaTEM and blaCTX-M genes.

Results: One hundred and ninety six K. pneumoniae isolates were collected from Imam Hussein hospital inTehran. Carriage of blaSHV, blaTEM and blaCTX-M genes was deteted by PCR and ESBL production was shown using the phenotypic confirmatory test (PCT). Overall, 160 isolates (81.6 %) carried blaCTX-M, 104 (53.06 %) had blaTEM and 82 (41.8%) harbored the blaSHV genes. ESBL production was observed in 92 isolates (46.9%) of which, 77 (83.6 %) harbored blaCTX-M, 57 (61.9 %) had blaTEM and 43 (46.7%) carried blaSHV. Finally, 7 (7.6 %) did not harbour any of the 3 genes and 24 (26.08%) carried all 3. Of the 104 ESBL negative isolates, 83 (79.8 %) harbored blaCTX-M, 47 (45.1%) had blaTEM, 39 (37.5%) carried blaSHV, 8 (7.7%) lacked all 3 genes and 17 (16.3%) carried all three.

Conclusion: Our study showed that ESBL phenotype did not correlate with gene carriage. In addition, blaCTX-M was dominant in our urinary Klebsiella pneumoniae isolates followed by blaTEM and blaSHV, respectively.

Keywords: Klebsiella pneumoniae, urinary isolates, extended- spectrums β –lactamases, ESBL

P870: **First report on qepA gene carriage in urinary isolates of Klebsiella pneumoniae in Iran**

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Background and Aim: The widespread use of quinolones for treatment of a variety of infectious diseases has resulted in development of bacterial resistance to these antibiotics. The novel plasmid-mediated fluoroquinolone efflux pump gene, qepA, was recently detected in clinical isolates of Enterobacteriaceae. The QepA efflux pump is a member of the major facilitator super family and confers reduced susceptibility to hydrophilic fluoroquinolones such as ciprofloxacin and norfloxacin. The aim of the present study was to investigate the presence of qepA gene in urinary clinical isolates of Klebsiella pneumoniae.

Methods: One hundred and ninety six K. pneumoniae isolates were collected from Imam Hussein Hospital in Tehran. The presence of qepA gene was detected by PCR using specific primers. E. coli TOP 10/pIP1206 harboring the qepA1 gene, kindly provided by Dr. Thomas Guillard (university of Reims, France), was used as the positive control.

Results: The results showed that of the 196 isolates, 30 (15.3%) were positive for qepA gene showing the expected amplification product of 403 bp.

Conclusion: We believe that this is the first report on the presence of qepA gene among clinical isolates of K. pneumoniae in Iran

Keywords: Klebsiall pneumoniae, qepA, urinary infection

P871: **Antibiotic resistance study of Staphylococcus aureus strains isolated from blood cultures of hospitalized heart disease patients in Shahid Madani Hospital of Tabriz**

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Background and Aim: Nosocomial infection is one of the complications of hospitalization and Staphylococcus areus (SA) is one of the most common and important microorganisms causing these infections. Increasing SA resistance to antibacterial drugs is one of the major health concerns, therefore study of the antibiotic resistance of SA is very important and it has a main role in preventing creation of resistant strains.

Methods: This investigation was a retrospective study and it was done by studying of 513 hospitalized heart disease patients' blood culture records in Tabriz Shahid Madani Hospital during a one-year period.

Results: Microorganisms in 71 cases (%14) of blood cultures grew. Eighteen point three percent of positive cultures were related to SA. Four cases (%31) of SA positive patients were females whereas 9 cases (%69) were males and the age average of patients was over than 40 years. The most of resistant cases (%31) were related to methicillin and ceftriaxon.

Conclusion: This study showed that there are methicillin resistant strains that probably related to malapropos prescription of ceftriaxone, therefor care and control must be performed on these bacteria together with avoiding unnecessary prescription of antibiotics because this affair can create and increase drug resistant strains.

Keywords: Staphylococcus areus, antibiotic resistance, blood culture

P872: **Idetentification of antigenic regions of a protein so as to enhance probiotic bacteria's quality**

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Background and Aim: Clostridium difficile is an anaerobic, sporogenous, rod-shaped gram positive bacterium which produces two important toxins, called Toxin A and Toxin B, which are common factors of causing Antibiotic-Associated Diarrhea (AAD). It is also the most common factor for causing diarrhea in patients admitted to hospitals for a long time. This bacterium can also lead to Pseudomembranous colitis. Use of antibiotics by patients affects their intestinal flora, and by long term consumption of these medicines, ideal environment would be created for Clostridium difficile growth. Growth and colonization of this bacterium leads to toxin production which would be released in intestinal. These two released toxins would attach to intestinal cell membranes and by preventing their natural function would cause inflammation and scar in intestinal. These toxins are among large toxins with high molecular weight. If we could produce just the immunogenic areas of them, we might be able to ready immune system against them. To do so, immunogenic components of these toxins can be identified and produced in bacteria like probiotics and through which improve probiotics function.

Methods: Using identifier software for immunogenic regions Antigenic peptides are determined using the method of Kolaskar and Tongaonkar (1990) Predictions are based on a table that reflects the occurrence of amino acid residues in experimentally known segmental epitopes. Segments are only reported if they have a minimum size of 8 residues. The reported accuracy of method is about 75%.

Results: Using identifier software for immunogenic regions showed us that there are many immunogenic regions in toxins A and B which can be used for experimental studies.

Conclusion: production of immunogenic regions in probiotic bacteria can have many advantages such as construction recombinant probiotic bacteria. Moreover, through creating recombinant adjuvant proteins accompanying these recombinant components, we might be able to increase their effects.

Keywords: Clostridium difficile ,probiotic bacteria,Toxin A, Toxin B

P873: **ANTIFUNGAL EFFECT OF ROYAL JELLY ON CANDIDA ALBICANS**

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Background and Aim: Royal jelly is a honey bee secretion that is used in the nutrition of larvae and adult queens. It is secreted from the glands in the hypopharynx of worker bees, and fed to female larvae in the colony. Royal jelly is secreted from the glands in the heads of worker bees, and is fed to all bee larvae, whether they are destined to become drones (males), workers (sterile females), or queens (fertile females). Royal jelly is collected and sold as a dietary supplement for humans, claiming various health benefits because of components such as B-complex vitamins such as pantothenic acid (vitamin B5) and vitamin B6 (pyridoxine). The overall composition of royal jelly is 67% water, 12.5% crude protein, including small amounts of many different amino acids, and 11% simple sugars (monosaccharides), also including a relatively high amount (5%) of fatty acids. It also contains many trace minerals, some enzymes, antibacterial and antibiotic components, and trace amounts of vitamin C,[2] but none of the fat-soluble vitamins, A, D, E and K. Royal jelly is used as a component in some skin care and natural beauty products. In holistic healing circles and popular alternative medicine folklore, royal jelly is believed to have anti-aging properties stemming primarily from its amino acid content and broad spectrum of vitamins and minerals.

Methods: In this research Inhibition of Candida albicans growth exposed to the local royal jelly was studied. The disc diffusion method was used to evaluate the zone of fungal growth inhibition using various concentrations of the royal jelly. The nystatin was used as a positive control and solvent distilled water was used as a negative control. Minimal Inhibitory Concentration (MIC) and Minimal Fungicidal Concentration (MFC) of the jelly and controls were determined and compared with each other.

Results: The results of this research showed that royal jelly in 400 µg/ml and 200 µg/ml concentrations completely were inhibited the growth of Candida albicans. The Minimal Inhibitory Concentration (MIC) and Minimal Fungicidal Concentration (MFC) were 25 µg/ml and 50 µg/ml respectively. The Inhibition zone diameter in 25 µg/ml, 50 µg/ml and 100 µg/ml were 14 mm, 29 mm and 46 mm respectively.

Conclusion: According to the this results , we conclude that royal jelly can be an important alternative medication against Candida albicans.

Keywords: Antifungal activity, Candida Albicans, Royal jelly

P874: **Isolation of effective bacteriophages against hospital E. coli**

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Background and Aim: Bacteriophages (also called “phages”) are viruses that kill bacteria. Phages are natural antimicrobial agents to fight bacterial infections in humans, in animals or in crops of agricultural importance. In antibiotic resistant bacteria, phages can be a good alternative. In this study we want to isolate phages from wastewater which can destroy hospital E. coli.

Methods: Water samples from environment were amplified by inoculating host E. coli to water and incubating in 37°C overnight. Then it was centrifuged after adding chloroform. The supernatant was exposed to the host bacteria in double-agar layer plates and plaques were observed.

Results: From this study, a total of six bacteriophages have been successfully isolated by using three different strains of hospital E. coli as a host. Host ranges of these phages were different. One of them kills three hosts and two others kill only one strain.

Conclusion: According to these observations, phages were capable of killing hospital bacteria such as E. coli. Phages, specially the one which was broad ranged, can be a good choice for phage therapy.

Keywords: bacteriophages, phage therapy, hospital E. coli

P875: **THE SURVEY OF ANTIBACTERIAL EFFECT OF ETHANOLIC AND METHANOLIC EXTRACT OF ZINGIBER OFFICINALE**

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Background and Aim: Zingiber officinale has particular place in Iran and China custom medicine from ancient. The prevalence of antibiotic resistance in bacteria is one of the main troubles in recent decades that the using of natural replacements with less side effects has been so noteworthy [2]. The purpose of present study survey of antibacterial effects of Z. officinale alcoholic extract is.

Methods: Antibacterial effects of ethanolic and methanolic extracts of Z. officinale were assessed in different density 50 to 400 mg/ml per disc diffusion method against Staphylococcus aureus, Bacillus subtilis, Escherichia coli and Pseudomonas aeruginosa.

Results: . Growth inhibitory results impressed that both of extracts have had efficient effects on positive and negative bacteria at high density and ethanolic extract has the best effect on P. aeruginosa. Indeed both of them have had fine effect on under experiment positive bacteria.

Conclusion: Due to gutted results from present survey Z. officinale can be used for antibiotic replacing particularly against Ps. aeruginosa that have a great resistance against of extensive numbers of antibiotics and S. aureus and also in can be used as permissive additive because of edible ability to increasing nutrients products maintains period.

Keywords: Z. officinale, Custom medicine , Alcoholic extract ,Antibiotic resistance

P876: **DNA Damage in Peripheral Blood Leukocytes of Infertile Men in North of Iran**

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Background and Aim: Male infertility constitutes the primary cause of infertility for up to 20% of couples between many other reasons. Genetic disorders are too important because DNA integrity in sperm and blood are necessary for having healthy next generation. The aim of this study was to evaluate the baseline DNA damage in blood samples obtained from normal and three groups of infertile individuals

Methods: DNA damage in blood (leukocytes) of fertile (n=10) and infertile (n=60) individuals were measured by alkaline single cell gel electrophoresis (comet assay). DNA damage in each group was calculated following visual observation and grading of comets under a fluorescence microscope. A positive correlation was observed between the DNA damage of leukocytes and infertility.

Results: Infertile mens (oligospermic, oligoasthenospermic and asthenospermic patients)showed various degrees of DNA damage significantly different from normal.

Conclusion: These results demonstrate that men with oligozoospermia and oligoasthenozoospermia have more DNA damage compared with normal mens in their leukocytes. DNA damage in infertile individuals is found to be higher than normal.

Keywords: Male infertility, Comet assay, DNA damage, Leukocyte

P877: **Identification of Pseudomonas aeruginosa isolates producing SHV-type extended-spectrum Betalactamase among hospitalized patients in two teaching hospitals, Sanandaj**

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Background and Aim: Pseudomonas aeruginosa is one of the most important causes of nosocomial infections and can acquire resistant to many antibiotics, including β-lactams. The aim of this study was to determine the distribution and antibiotic susceptibility patterns of extended-spectrum beta-lactamase (ESBL) type SHV producing isolates from hospitalized patients in two teaching hospitals, Sanandaj, Iran.

Methods: A total of 123 P. aeruginosa were isolated from various clinical specimens. The double-disk synergy test was performed on the isolates for the detection of ESBL. SHV gene was confirmed by PCR method.

Results: The most resistant antibiotics tested against P. aeruginosa were ceftazidime (23.58 %) and cefotaxime (30.48%). All the P. aeruginosa isolates were tested for ESBL production and 22 (17.89%) were found to be ESBL producers. Out of 123 strains, 12 (10.57%) were SHV positive. Multiple resistances antibiotics were often associated with ESBL-producing organisms. Nosocomial infection (OR=2.14), days of hospitalization (OR=14.34), ICU hospitalization (OR=3.4), presence of catheter (OR=3.63), use of antibiotics within previous two weeks (OR=5.51) and use of ventilator (OR=3.75) were risk factors for pseudomonas nosocomial infection SHV positive ESBL.

Conclusion: The high relatively of SHV producing bacteria in nosocomial agents of concern their potential spread among patients. The increasing risk factors for spreading of SHV enzyme were nosocomial infection, days of hospitalization, ICU hospitalization, presence catheter and use of antibiotics

Keywords: P. aeruginosa, Antibiotic resistance, Extended-Spectrum beta-Lactamase, SHV gene

P878: **Frequency and antimicrobial susceptibility patterns of bacterial pathogens isolated from septicemic patients in Imam khomeini Hospital in Kermanshah, Iran, 2012**

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Background and Aim: Septicemia is one of the life threatening infections. Bacteria are responsible for majority of septicemia in hospitals. In this study we aimed to assess the bacterial agents of septicemia and their antibiotic susceptibility pattern in blood sampled from patients admitted in Imam Khomeini hospital in Kermanshah in 2012.

Methods: In this study, 3624 blood samples were assessed. They were cultured in TSB (Trypticase Soy Broth) and incubated up to 3 weeks. The suspected samples for infection were subsequently cultured on BA (Blood Agar), EMB (Eosin Methylene Blue Agar) and other specific media. Finally bacteria were identified using standard bacteriological method. Antimicrobial susceptibility testing were done using disk diffusion method according CLSI standards. Data analysis was performed using SPSS software.

Results: Of 3624 blood samples cultured, 70 cases (1.9%) were positive for bacterial agents, comprised 36 cases (51.5%) isolated from men and 34 cases (48.5%) isolated from women. The most common bacteria isolated were Citrobacter diversus (31.4%), Staphylococcus aureus (28.5%) and Pseudomonas aeruginosa (12.8%), respectively. The results of antimicrobial susceptibility testing showed the lowest resistance was for imipenem, ciprofloxacin and amikacin and the highest resistance belonged to ceftazidime and cephalothin.

Conclusion: Results of this study showed that bacteria isolated all belonged to nosocomial infectious agents, following the infection control protocols in hospitals can reduce the frequency of blood infections.

Keywords: blood culture, septicemia, antimicrobial susceptibility testing

P879: **The frequency and antibiotic susceptibility pattern of bacterial agents isolated from urinary tract infections in children admitted in Dr.Mohamad Kermanshahi hospital, Kermanshah, 2011-2012**

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Background and Aim: The urinary tract infection (UTI) is one of the most common infections which occur in various ages in particular in children and may have dangerous consequences. On the other hand, the bacterial agents of UTI and their susceptibility are continuously changing in different parts of the world. This study aimed to study the bacterial agents of UTI and their susceptibility to common antibiotics in children in 2011-2012.

Methods: In this study the midstream samples of urine of 6547 children who admitted by the Dr.Mohamad Kermanshahi hospital in Kermanshah city were collected and cultured on specific and differential media aseptically. Isolated bacteria were diagnosed by bacteriological tests. Then the susceptibility of bacteria was assed using disk diffusion method.

Results: Out of 6547 urinary cultures, 366 cases (5.6%) were positive for bacterial infections. The most common bacterial agents were: Escherichia coli 242 cases (66%) cases, Staphylococcus saprophyticus 51 cases (14%), Citrobacter diversus 45 cases (12.2%), Citrobacter frundi 16 cases (4.35%), Entrobacter aerogenese 9 cases (2.45%) and Pseudomonas aeroginusa 3 cases (0.8%). The results of antimicrobial susceptibility testing for E.coli showed that the highest resistance rate were against to ampicillin (91.4%) and gentamicin (79.4%) and the lowest resistance rate were against to nitrofurantoin (47.3%).

Conclusion: This study indicated E.coli is the number one bacterial agent for UTI in children and it has become resistant to many first line antibiotics. So it is recommended that the urinary isolates of E.coli be tested for antibiotic susceptibility to select the appropriate antibiotics for treatment.

Keywords: Antibiotic susceptibility pattern, urinary tract infection, Escherichia coli

P880: **New trends in diversity and frequency of diarrheagenic bacteria among children with diarrhea in Tehran, Iran**

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Background and Aim: Diarrheal diseases are a leading cause of morbidity and mortality among young children in low-income countries. Causes of diarrhea in areas of endemicity include a wide variety of bacteria, viruses, and protozoa. Among the bacterial agents, the five most important are Shigella, Salmonella, Escherichia coli, Yersinia, and Campylobacter. The aim of this study is to investigate frequency of common and uncommon enteropathogenic bacteria in hospitalized children with diarrhea

Methods: A total of 285 diarrheal stool samples were obtained during one year study in 2012. All children were under 10 years olds who were admitted to Children's Medical Center with acute diarrheal diseases. Macroscopic and microscopic features of their samples were analyzed and the samples were cultured for identification of responsible pathogens on specific media.For detection of enteropathogenic Escherichia coli were used PCR method. In the case of Clostridium difficile, both methods of PCR on the grown colonies and ELISA on the stool samples were used. The presence of fecal leukocytes in stool samples was used as an indicator of inflammatory diarrhea.

Results: Direct examination of the stool samples showed frequency of inflammatory diarrhea in amount of 29.% among these patients. Among a total of 285 studied samples, Campylobacter spp. (0.7%),Aeromonas spp. (0.7%), Shigella flexneri (0.7%),Salmonella spp. (0.7%), enteropathogenic Escherichia coli (2.1%), and C. difficile (1.7%) were detected as responsible pathogens for diarrheal diseases in these patients.

Conclusion: Pathogenic bacteria were found to be significant causes of acute diarrhea in children. Low frequency of common pathogenic bacteria and presence of rare enteropathogens among the patients' samples with inflammatory diarrhea proposed change in etiology of this disease among diarrheatic children in Tehran, Iran.

Keywords: diarrheagenic bacteria ; enteropathogenic Escherichia coli: Clostridium difficile

P881: **Study of Antibacterial Activity of Melittin derived from Iranian Honey Bee Venom on clinical isolates of Pseudomonas aeruginosa**

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Background and Aim: Infections caused by Pseudomonas aeruginosa has been reported from all parts of the world. As it is an opportunistic bacteria, in cases where the immune system is weakened (diabetes, cancer, burns, etc), invades the body and it is very difficult to control these kind of infections due to antibiotic resistance. Inappropriate use of antibiotics against pseudomonas infection accompany to natural resistance led to antibiotic-resistant species. According to this issue, tracing for new types of antibiotics is necessary. During the recent decades, many antimicrobial peptides have been isolated from natural sources including venomous animals, insects, plants, human, invertebrates and etc. Antimicrobial peptides may be an alternative to be used instead of antibiotics in the near future. Honey bee as a natural source proved to has an efficient antibacterial peptide designated melittin. The objective of current study was purification of melittin from honey bee venom and evaluation of its antibacterial activity against clinical isolates of Pseudomonas aeruginosa.

Methods: Bee venom collected from a hive corresponding to Apis melifera meda in Chaharmahal-Bakhtiari, Shahrekord using a venom collector based on Benton protocol with some modifications, transferred to Pasteur Institute of Iran and maintained at freezer for further analysis. The venom prepared in demonized water and protein concentration estimated using BCA method. SDS-PAGE was performed to control the quality of venom. Prepared bee venom injected to C18 column and eluted with a linear gradient of acetonitrile in 0.1 TFA for 55 minutes using Reverse Phase high performance liquid chromatography (RP-HPLC) technique. The eluted fractions monitored at 214 and 280nm, collected from the column, and lyophilized in a freeze dryer. Pseudomonas aeruginosa isolated from different hospitalized patients with different Pseudomonas infections. Minimum Inhibitory Concentration (MIC) of melittin examined by microdilution broth method based on NCCLS recommendation with some modification. Minimum Bactericidal Concentration (MBC) obtained by colony count in muller hinton agar.

Results: SDS-PAGE showed that isolated melittin was highly pure. The MIC50 for all isolates (n=48), mucoid colonies (n=21), non-mucoid colonies (n=27) were 16.47, 6.25, and 12.5 ?g respectively. MIC50 for tracheal, urine and cystic fibrosis isolates were 20.73, 18.62, and 7.68 ?g respectively. Total MIC/MBC ratio was 0.44.

Conclusion: It was found that melittin had a significant inhibitory effect on the growth of Pseudomonas aeruginosa isolated from different patients. According to results, melittin is a potent anti-pseudomonas agent. Interestingly, the mucoid colonies were more sensitive to melittin than non-mucoidal. This finding is a new hope for treatment of Pseudomonas aeruginosa infections in cystic fibrosis patients. This study is pending to evaluation of antibacterial activity in a greater population and different bacterial species.

Keywords: Honey Bee Venom, Melittin, Pseudomonas aeruginosa, RP-HPLC, MIC, MBC

P882: **Antifungal activity of methanolic extract of Hypericum species from Iran**

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Background and Aim: The genus Hypericum is one of the most consumed medicinal plants all over the world. The aim of present study was to investigate antifungal activity of the methanolic leaf extract of Hypericum vermiculare, H. lysimachioides and H. perforatum against Candida albicans.

Methods: The leaves of the three Hypericum species were dried and powdered. The methanolic extracts were obtained by soxhlete apparatus. Antifungal activity of the extracts against C. albicans was studied by disc diffusion method. The prepared discs with extract concentration of 4000 µg/ml were placed on Muller Hinton agar plates. The plates were incubated at 35 ºC for 24 h. Diameters of fungal growth inhibition zones (mm) were measured and recorded.

Results: The results showed that methanolic extracts of H. perforatum and H. vermiculare leaves had inhibitory effects on C. albicans with inhibition zones of 4.0-5.0 mm. H. lysimachioides had no obvious effect on the fungal growth.

Conclusion: Our results indicated that methanolic extracts of leaves of H. vermiculare and H. perforatum inhibited the growth of C. albicans and therefore, they can be considered as potential antifungal agents for treating of Candida-related mycoses.

Keywords: Hypericum; Candida albicans; Metanolic extract; Antifungal activity

P883: **Evaluation of Induced Antibodies Responses Against E.coli O157: H7 OPS by Its Conjugate to Pseudomonas aeruginosa Recombinant Exotoxin A**

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Background and Aim: E.coli O157: H7 is one of the most important causes of bloody diarrhea. Hemorrhagic colitis cause hemolytic uremic syndrome (HUS), which causes the syndrome of acute renal failure ,microangiopathic hemolytic anemia and thrombocytopenia. The aim of present study is immunological evaluation of E.coli O157: H7 detoxified OPS - Pseudomonas aeruginosa Recombinant Exotoxin A conjugate in mice as a candidate vaccine.

Methods: OPS of this bacteria extracted by hot phenol method, then dialysis and electerophoresis were done.To improve immunogenicity, the purified antigen was coupled to recExoA with ADH as a spacer and EDAC as a linker. The reaction mixture was passed through a sepharose CL-2B column. Sterility and toxicity tests were shown that prepared conjugate was non-toxic and non-pyrogenic. Then four group of female BALB/c mice (each group was included 15 mice) was selected. Vaccination was performed by three doses with two week interval. Then serum samples was collected and antibody responses was measured by ELISA method for total IgG, IgG1, IgG2a, IgG2b, IgG3, IgM and IgA.

Results: Two week after first dose of vaccination there was no significance different antibody titers between groups that were immunizided by OPS157and OPS157-recExoA. But after second and third doses, OPS E.coli O157 H7-recExoA showed significance increasing in all types of antibodies titers in versus OPS157. Overall results of anti LPS inductions for total IgG, IgM, IgA, IgG1, IgG2a, IgG2b, and IgG3 were shown:: OPS E.coli O157 H7-recExoA> OPS E.coli O157 H7 > recExoA.The anti LPS IgG antibody was predominantly of IgG1 subtype and then IgG3, IgG2a, IgG2b, respectively.

Conclusion: These results indicated that OPS from E.coli O157: H7 increase anti OPS antibodies in conjugate form with Pseudomonas aeruginosa recExoA and can be an appropriate effective candidate vaccine for this bacteria.

Keywords: E.coli O157: H7, OPS, recExoA, conjugate, ELISA

P884: **Evaluation of Induced IgG Subclasses by OPS E.coli O157H7 Conjugate to Pseudomonas aeruginosa Recombinant Exotoxin A against E.coli in mice**

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Background and Aim: Patients with strains of E. coli O157: H7 are bloody diarrhea, a dangerous and threatening complication called hemolytic syndrome are uremic.In this study is immunological evaluation of E.coli O157: H7 detoxified OPS - Pseudomonas aeruginosa Recombinant Exotoxin A conjugate in mice as a candidate vaccine.

Methods: After mass culture, LPS Sequestration by modified hot phenol and OPS was extracted with dialysis .To improve immunogenicity, the purified antigen was coupled to recExo Awith ADH as a spacer and EDAC as a linker. The reaction mixture was passed through a sepharose CL-2B column. Then four group of female BALB/c mice (each group was included 15 mice) was selected . Vaccination was performed by three doses with two week interval. Then serum samples was collected and antibody responses against OPS was measured by ELISA method for total IgG, IgG1, IgG2a, IgG2b, IgG3, IgM and IgA .

Results: Two week after first dose of vaccination there was no significance different antibody titers between groups that were immunizided by OPS157and OPS157-recExoA. But after second and third doses, OPS E.coli O157 H7-recExoA showed significance increasing in all types of antibodies titers in versus OPS157. Overall results of anti LPS inductions for, IgG1, IgG2a, IgG2b, and IgG3 were shown: OPS E.coli O157 H7-recExoA> OPS E.coli O157 H7 > recExoA. The anti LPS IgG antibody was predominantly of IgG1 subtype and then IgG3, IgG2a, IgG2b, respectively.

Conclusion: These results indicated that OPS from E.coli O157: H7 increase anti OPS antibodies in conjugate form with Pseudomonas aeruginosa recExoA and can be an appropriate effective candidate vaccine for this bacteria.

Keywords: E.coli O157: H7, OPS, recExoA, conjugate, ELISA

P885: **The relation of HLA-DRB1 and viral infection (HBV, HCV, CMV) in kidney transplant recipients**

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Background and Aim: Viral infections and genetic factors such as HLA, especially HLA-DRB1 molecules may affect the kidney transplant outcome. The correlation between these alleles and viral infections is more important. In the present study, the relation of HLA-DRB1 alleles and viral infections (Cytomegalovirus and Hepatitis B and C) in the kidney transplant has been studied.

Methods: In this study, 69 kidney transplant recipients were clinically studied. 41 out of 69 of patients were investigated using HLA Typing. HLA-DRB1 typing was performed using PCR-SSP method according to the kit manufacturer instruction (BAG, Germany). PCR products were analyzed by electrophoresis on a 2% agarose gel and the results were interpreted using BAG typing software (BAG, Germany) and re-checked manually by typing worksheet. Moreover infections with three important and common viral infections in kidney transplant recipients including HBV, HCV and CMV were also determined using ELISA and molecular method. Also Epi Info software was used for statistical analysis.

Results: Among 69 samples, viral infection was found in 8 of 28 (29%) in acute rejection group and 7 of 41 (17.1%) patients in non rejection group after transplantation. Statistical analysis indicated that there is not any relation between HCV, HBV or CMV infection and kidney transplant acute rejection ($P>0.05$). Analysing the relationship between each studied infection and alleles frequencies indicated that HLA-DRB1*07 alleles may be associated with hepatitis C virus infection ($P<0.009$)

Conclusion: However, the results of present study did not showed any relation between viral infection and rejection in the short time after kidney transplant. It can be due to the low number of patients. Considering the significant role and susceptibility to renal diseases or viral infections, HLA-DRB1 typing for both recipients and donors is highly recommended.

Keywords: HLA-DRB1, Cytomegalovirus, Hepatitis B, Hepatitis C, kidney transplant

P886: **Prevalence of the 8 genes encoding P fimbriae in uropathogenic Escherichia coli clinical isolates**

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Background and Aim: Uropathogenic E.coli (UPEC) show different virulence factors, such as adhesins, invasins, toxins, sidrophors and genes required for capsular biosynthesis. P fimbriae, the virulence factor of Escherichia coli second common virulence factor of UPEC, which plays an important role in the pathogenesis of ascending UTIs and pyelonephritis in humans and encoded by the pap (pyelonephritis associated pili) gene cluster found on the chromosome.: Investigation eight pap genes: A, EF, G (internal and flanking alleles I,II and III) and C in uropathogenic Escherichia coli.

Methods: One hundred thirty eight (138) uropathogenic E. coli (UPEC) clinical isolates were investigated in this study. In addition, 30 E. coli isolates collected from feces of healthy humans were used as control. The PCR assays were performed in a final volume of 25 µL, containing DNA (2 µL boiling lysate), 2 µL MgCl2 , 2 µL dNTP, 1U Taq Polymerase, 1X PCR Buffer and 0.6 µM primers except primers of papG internal allele I and C genes which were used at a concentration of 0.3 µM. PCR condition was set up as follows: 94°C for 3 min followed by 30 cycles of 94°C for 45s, 63°C for 30s, 72°C for 2 min and 10 min at 72°C as final extension. Chi square test or Fisher's exact test was used to compare the occurrence of virulence markers in cases and controls. Results were considered as statistically significant at $P < 0.05$.

Results: From 138 UPECs, (19.5%)27, (21.01%)29, (11.5%)16, (6.9%)9 (21.7%)30, (1.4%)2, (3.6%)5 and (10.1%)14 and from 30 human Commensally E. coli, (0%)0, (3.3%)1, (6.6%)2, (16.6%)5, (0%)0, (0%), (3.3%)1 and (0%)0 isolates presented papA, papEF, papG internal allele III, papC, papG internal allele I, papG internal allele II, papG allele I flanking and papG alleles II & III respectively.

Conclusion: Prevalence of the papA and papEF genes in UPEC isolates were statistically more significant than those belonging to Flora groups ($P \leq 0.001$) but Prevalence of the other pap genes was not differences statistically significant ($P > 0.05$).

Keywords: Uropathogenic E.coli, virulence factor, P fimbriae, pap genes

P887: **Antimicrobial activity of camel's milk against pathogenic strains of Escherichia coli O157: H8 and Klebsiella pneumoniae with multidrug resistance**

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Background and Aim: Camel's milk has supported Bedouin, nomad and pastoral cultures since the domestication of camels millennia ago. Herders may for periods survive solely on the milk when taking the camels on long distances to graze in desert and arid environments. Camel milk has a high vitamin and mineral content and immunoglobin content. The composition of camel milk depends on its feed and species.

Methods: The inhibitory activity of camel's milk and colostrum at 4°C and 20°C was tested by the well diffusion assay against six pathogens. The activity was also studied in situ by monitoring the growth of a three-strain mixture of Klebsiella pneumoniae or Escherichia coli O157: H8 in camel's milk, colostrum or Tryptic Soy Broth (TSB) as function of time.

Results: The results of the well diffusion assay show that Bacillus cereus was resistant to the inhibitory activity present in camel's milk and to the colostrum, while Klebsiella pneumoniae and Escherichia coli O157: H8 were the most sensitive as judged by the diameters of the inhibition zones. The in situ test revealed a typical inhibition pattern of both pathogens in camel's milk samples during 48 h of incubation at both storage temperatures. The colostrum exerted a definite bactericidal activity against Escherichia coli O157: H8 at ambient temperature, and the viable counts decreased to below the detectable level in a 1-mL sample at 48 h, while at the refrigeration temperature, the counts were reduced by 1 and 3 log units compared to the initial inoculum and to the positive control, respectively.

Conclusion: The camel's milk and colostrum samples had a bacteriostatic effect against Klebsiella pneumoniae during the first 8 h of incubation; thereafter, a tendency to increase was noted in the colostrum at 20°C. Similar experiments were carried out on Escherichia coli O157: H8 in heat-treated camel's or cow's milk and showed that the inhibitory effect of camel's milk was reduced by heat treatment.

Keywords: Escherichia coli O157: H8, camel's milk, colostrum, Klebsiella pneumoniae

P888: **Investigation of the Activity of Camel Milk Casein against Hepatitis B Virus**

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Background and Aim: Hepatitis B is an infectious inflammatory illness of the liver caused by the hepatitis B virus (HBV) that affects hominoidea, including humans. Originally known as serum hepatitis. the disease has caused epidemics in parts of Asia and Africa, and it is endemic in China. About a third of the world population has been infected at one point in their lives, including 350 million who are chronic carriers. Several vaccines have been developed for the prevention of hepatitis B virus infection. These rely on the use of one of the viral envelope proteins (hepatitis B surface antigen or HBsAg). The vaccine was originally prepared from plasma obtained from people who had long-standing hepatitis B virus infection. However, it is made using a synthetic recombinant DNA technology that does not contain blood products. One cannot be infected with hepatitis B from this vaccine. Therefore, discovery and identification of a new drug for HBV treatment is a high priority. Camel milk is a traditional medicine that could improve the control of HBV.

Methods: Casein was purified from defatted camel milk to electrophoretic homogeneity. PBMCs and HepG2 and HeLa cell lines were used. Three kinds of experiments were conducted. HBV was directly interacted with casein and then mixed with different cell types, casein was incubated with the cells and then exposed to HBV, and the HBV pre-infected cells were treated with casein at different concentrations and time intervals. Non-infected cells were used to assess cytotoxicity and the apoptosis effect of casein.

Results: Direct interaction of casein (with or without ?-lactalbumin) with neither the virus nor the cells prevented HBV cell entry. However, casein with ?-lactalbumin induced a cytotoxic effect in HepG2 and HeLa cell lines but not in human naïve leukocytes. At all concentrations tested, casein with ?-lactalbumin could induce apoptosis in both infected and non-infected HepG2 cells.

Conclusion: Camel milk casein (with or without α-lactalbumin) did not demonstrate any anti-HBV activity. However, the cellular apoptotic cascade was initiated in HepG2 and HeLa cells treated with casein (with α-lactalbumin) but not in naïve leukocytes.

Keywords: Camel, Casein, Hepatitis B virus

P889: **Induction of IgG and IgM antibodies by E.coli O157: H7 detoxified LPS conjugate with diphtheria toxoid in mouse**

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Background and Aim: Infections caused by E.coli O157: H7 may lead to severe diarrhea and hemorrhagic colitis with complications such as microangiopathic hemolytic anemia, thrombocytopenia, and fatal acute renal failure, which are summarized as hemolytic uremic syndrome (HUS). Lipopolysaccharide (LPS) is a key factor in virulence as well as both innate and acquired responses that is used in conjugate form to enhance the immunogenicity against this infection. The purpose of this study is Induction of IgG and IgM antibodies by E.coli O157: H7 detoxified LPS (D-LPS) - diphtheria toxoid (DT) conjugate in mice.

Methods: LPS of this bacteria extracted by hot phenol method, then dialysis and electerophoresis were done. The purified LPS then detoxified by alkaline method. To improve immunogenicity, the purified antigen was coupled to DT with ADH as a spacer and EDAC as a linker. The reaction mixture was passed through a sepharose CL-2B column. Sterility and toxicity tests were done. Then four group of female BALB/c mice were immunized with D-LPS-DT, D-LPS, DT and normal saline as control group .Vaccination was performed by three doses with two week interval. Then serum samples was collected and antibody responses against LPS was measured by ELISA method for total IgG and IgM.

Results: Extraction with LAL method was tested for pyrogeny and toxicity testing and non-pyrogenesis and non-toxicity were approved. Two week after first dose of vaccination there was no significance different antibody titers between groups that were immunizided by LPS and LPS-DT. But after second and third doses, LPS-DT showed significance increasing in antibodies titers in versus LPS ($p<0/01$).

Conclusion: Overall results of anti LPS inductions for total IgG and IgM were shown: LPS-DT>LPS >DT. Conjugate form elicited a significant rise in IgG and IgM. The levels of anti LPS IgG at 2 week after second injectin was higher than IgM. These results indicated that LPS from E.coli O157: H7 increase anti LPS IgG and IgM antibodies in conjugate form with diphtheria toxoid.

Keywords: E.coli O157: H7, LPS, DT, conjugate

P890: **Identification of the various species of Leptospira in the environmental soil and water samples obtained from the native regions in the Northern parts of Iran.**

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Background and Aim: Leptospirosis is one of the most significant common diseases between the human and animal which is prevalent throughout the world. This disease is to be construed as a native disease in the Gilan and Mazandaran provinces and the risk of the infection with the strains of pathogen is high.

Methods: 70 samples of water and soil were collected from the suburbs of the Tonekabon Township located in the northern parts of Iran in the spring of 2012. In order to isolate the Leptospira spp from the water and soil, the membranous filters with two different pores were employed. Then the filtered liquid was inoculated in/ against the EMJH medium and incubated in the 30℃ for 1 month. After the enrichment, bacterium's DNA was extracted by the phenol chloroform method. In order to diagnose pathogenic spp and saprophytic spp of the Leptospira, duplex-PCR hybridization and 16S rRNA and Lip32 primers were used.

Results: Of total 70 samples of collected water and soil with the aid of duplex-PCR technique, 18 strains were identified out of which 16 strains were diagnosed as saprophyte and 2 strains as pathogen. Therefore, prevalence rate of this bacterium in the studied region was evaluated 25.7%.

Conclusion: The results obtained from this research suggest that duplex-PCR technique can be used to identify the Leptospira spp in the water and soil samples. Because of using the mentioned primers, this method is able to differentiate between the saprophytic and pathogenic Leptospira.

Keywords: Leptospira, water and soil samples, Northern part of Iran

P891: **The Cytomegalovirus Infection among Iranian kidney graft recipients**

Zakieh Rostamzadeh[1]

1- nariman sepehrvand

Background and Aim: Background: Cytomegalovirus (CMV) infection is one of the most common infectious problems following kidney transplantation. In this study we are aimed to investigate the CMV infection in the setting of renal transplant recipients in Urmia-Iran, using both ELISA and polymerase chain reaction (PCR) methods.

Methods: Methods: Ninety six renal transplant recipients were selected randomly and enrolled in a cross-sectional study. Blood sampling was done via venipuncture, and all sera were investigated for anti-CMV IgM, and the seropositive cases in association with 14 randomly selected seronegative cases were investigated with PCR assay.

Results: Results: Thirty three patients (34.3%) were seropositive for anti-CMV IgM, 3 patients (3.1%) were in borderline range, and 60 patients (62.5%) were seronegative. By considering the patients with borderline anti-CMV IgM levels as seropositive, 37.5% were seropositive for anti-CMV IgM. Among 36 seropositive cases, the CMV infection was confirmed in 19 (52.7%) of them using PCR. Age (P=0.40), educational status (P=0.77), History of pre-transplantation dialysis (0.52), History of Blood Transfusion (P=0.52), and immunosuppressive regimen were not statistically different among recipients with positive versus negative CMV PCR study results.

Conclusion: The Cytomegalovirus Infection among Iranian kidney graft recipients (using ELISA & PCR methods) Zakieh rostamzadeh , nariman sepehrvand, javad shirmohamadi Medical science urmia-iran Abstract Conclusion: The seroprevalence of CMV infection was demonstrated to be high in renal transplant recipients of Urmia-Iran. The rate was higher compared to several previous reports in the literature. ELISA method has an appropriate sensitivity to screen the recipients for CMV infection but considering its relatively low specificity, the seropositive cases are better to be confirmed by further PCR study.

Keywords: ELISA, IgM, PCR Cytomegalovirus, Renal Transplantation,

P892: **The seroprevalence of Parvovirus B19 among Kidney Transplant Recipients:**

Zakieh Rostamzadeh[1]

1- nariman sepehrvand -sima masoodi-javad shirmohamadi iran urmia

Background and Aim: Background: Parvovirus B19 is a DNA virus which is responsible for several various diseases in human. Parvovirus B19 induced persistent anemia is one of its manifestations which is relatively common in the setting of transplant recipients. This study was aimed to investigate the seroprevalence of parvovirus B19 among kidney transplant recipients

Methods: Abstract: . Methods: 91 transplant recipients were selected randomly, and were investigated for several variables including age, sex, educational status, history of hemodialysis (HD), history of blood transfusion, and immunosuppressive therapy. 2 cc of blood samples were collected via venipuncture and evaluated for anti-Parvovirus B19 IgG antibody using Enzyme linked immunosorbent assay (ELISA

Results: Results: All recipients were anemic, 72.5% of them suffering from severe anemia (Hb<=11 in men and <=10 in women). Sixty three patients (69.2%) were seropositive for parvovirus B19. There was no significant difference among the age, sex, educational status, history of blood transfusion, history of HD, and the immunosuppressive therapy of seropositive and seronegative groups

Conclusion:).. Conclusion: The seroprevalence of parvovirus B19 was relatively high in the setting of kidney transplant recipients. Anemia is a common problem in these patients and often remains under-treated. However our study failed to find a correlation between the severity of anemia and seropositivity of parvovirus B19.

Keywords: Keywords: Parvovirus B19, ELISA, Renal Transplantation, Anemia

P893: **The relationship between clinical symptoms and laboratory findings of urinary tract infection in children Hospitalization in jahrom Hospitals from 88 until 91.**

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Background and Aim: Urinary tract infections (UTI), the most common serious bacterial infections in infants and young children. 5% of girls and 1% of boys up to age 11 will experience it. Failure to timely diagnosis and treatment can lead to severe complications. The present study in order to investigate the relationship between clinical symptoms and laboratory findings of urinary tract infection in hospitalized children in the jahrom from 88-91 years.

Methods: This research is a descriptive study. The study population included all patients were hospitalized with a primary diagnosis (UTI). The records of 420 patients with active urine analysis, positive culture and clinical symptoms were examined. In this study variables of age, sex, crime findings, clinical symptoms, laboratory findings, circumcision, turn UTI and treatment were analyzed. Data were analyzed with SPSS software.

Results: A total of 420 patients, 276 cases were males and 144 cases females. 193 cases were Children under 4 years and 227 cases children 4-11 years old. Symptoms in children under 4 years were restlessness and impatience when urinating, frequent urination, fever and in children 4-11 years old dysuria, urinary frequency, urinary tract inflammation ،redness and odor urine. In 357 patients with clinical symptoms, UC and UA was positive. At 20 female and 12 male with clinical symptoms ,UC and UA was negative. 137 cases was used the catheter. 107 cases had been circumcised. 31 cases of children without clinical symptoms associated with UTI have positive UC in 28 of these cases have negative UA. In 317 patients infected with E.coli and 45 patients Klebsiella, and in 26 cases Proteus were reported. According to the antibiogram testing in 324 cases combined antibiotic ampicillin- gentamicin and in 64 patients ceftriaxone was prescribed.

Conclusion: As regards in 7.6% (32 cases), patients with a positive clinical symptoms results of culture and analysis were negative and in 7.4% (31 cases),clinical symptoms were negative and laboratory results were positive (31 cases UC positive and 28 cases UA negative) It could be concluded that it was essential for correct diagnosis cause of the disease ،clinical symptoms, urine culture and analysis tests should be performed together particularly in children under 4 years old .In cases where there were no symptoms and laboratory results were positive could be due to catheter infection, Sampling errors in females, laboratory error, is non-sterile specimen container. Experimental results obtained from antibiogram test in 83.5% of patients the combination antibiotic was ampicillin- gentamicin. that result was a significant positive.

Keywords: clinical symptoms- Urinary Tract Infection- children-jahrom

P894: **Comparison of Hand Washing with Surgical Scrub Brush in the Specified Time and Counting the Number of Hands Pulling on the Contamination of the Surgical Team in Jahrom Hospitals**

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Background and Aim: Surgical site infections are often caused by bacteria colonized on the skin near surgical incision. Hand washing is the most important method of preventing hospital infections. This study compared effect of hand washing method with surgical scrub in specified time and counting the number of brush usage on the contamination of hands in the surgical team of Jahrom hospitals

Methods: Fifty persons of surgical team were selected by random sampling and were divided into the two groups (scrub in the specified time and counting the brush numbers). Before washing, the fingertips of both groups were sampled with a swab dipped in normal Saline. Then, both hands put in the sterile glove containing 10cc nutrient broth for 20-30 seconds. All samples were prepared by dilution 1/1000 of environment cultured on nutrient, blood and MacConky Agar. After 24-48 h incubation, the samples in terms of number and type of bacteria were investigated.

Results: All samples of hands before washing were culture positive (100%). Counting the number of colonies before and after washing showed a significant difference (p=0.01).

Conclusion: Compared the results of both methods to wash, washing with brush has a greater impact on reducing bacterial load, but brush washing gave the negative impact on skin and it consumed more time and increased accumulation of microorganisms on the damaged skin.

Keywords: Hand hygiene- Surgical team, Surgical scrub, Drawing brush.

P895: **Effect of black seed powder the number and performance of monocytes in laboratory rats inoculated with visceral leishmaniasis**

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Background and Aim: Nigella is a native herbaceous plant belonging to the family of the Ranunculaceae or Buttercup. The seeds of this plant contain essential oils, various sugars, resinous material, albuminoide and a saponin is called melantin. Has been reported to black seed increase the proportion of helper T lymphocytes than suppressor T lymphocytes, and increased activity of natural killer cell. The observation black seed that increases the amount of IL-1,so black seed can affect the phagocytic cells and monocytes. This study aimed to investigate effect of black seed powder the number and performance of monocytes in laboratory rats inoculated with visceral leishmaniasis.

Methods: Black beans prepared and approved by the relevant experts and turned into powder using a standard rat diet at doses of 0.01, 0.1 and 1 g / kg body weight was mixed. 24 male rats Bal / c that were kept in standard conditions were selected. Before testing,for determine the number of blood monocytes, blood samples were taken on rats. Then the rat infected with injection 0.1 ml suspension of promastigotes of the parasitic strains L.infantum are containing 2 million parasites into rats tail. Then randomly divided into 4 groups, three groups received the same doses and a control group that received normal diet . After 5 weeks, from rats heart 3 ml blood sample was taken from a few drops use for peripheral blood smear to count monocytes and 2 ml were used in real time pcr .

Results: In the control group the number of monocytes before and after parasite inoculation respectively 3.1±1.2% and 4±1.7%, In the receptor dose 0.01 g / kg of black seed powder 3.5±1% and 4.1±1.2%, In the receptor dose of 0.1 g / kg of black seed powder3.3±1.6% and 4.9±1.1% , In the receptor dose 1 g / kg of black seed powder 3.2±1.7% and 5.1±1.3% was calculated. Parasitic load in first time injecting parasite in all groups was 2 million Leishmania promastigotes . After 5 weeks of parasitic load in control group 3.39×106 , In the receptor dose 0.01 g / kg of black seed powder2.81×106, In the receptor dose of 0.1 g / kg of black seed powder2.1×106, In the receptor dose 1 g / kg of black seed powder1.71×106,promastigotes were calculated. Results and statistical data shows that the use of black seed has significantly increased phagocytic monocytes($P<0.05$).

Conclusion: The results was significant increase in phagocytic monocytes. Although in the control group the number of monocytes was increased, but this increase is not significant and has not reduced the parasite load. But however is increased the Nigella dose the number of monocytes may also increased,this monocytes were functionally efficient because increased dose of black seed powder and monocyte percentage cause of decreased parasite load. Can be used the powder of Nigella to increase monocyte phagocytosis in diseases such as kala-azar to hasten healing of disease.

Keywords: L.infantum-rat-monocyte-black seed-jahrom-real time

P896: **Molecular Detection and Association of QnrA, QnrB, QnrS and BlaCMY Resistance Genes Among Clinical Isolates of Salmonella spp. in Community**

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Background and Aim: spread of resistance among diseases bacteria

Methods: Prevalence of three plasmid- mediated quinolone resistance determinant qnrA, qnrB, qnrS and extended spectrum Cephalosporins determinant blaCMY, among eighty five isolates of Salmonella spp. collected in the community for 2008 to 2010 were determined by PCR.

Results: Not only qnr genes but also bla genes were positive in twenty four of different isolates. PCR assay detected 22 of 85 (25.8%) Salmonella spp. carried the qnrA, 1 (1.17%) of 85 isolates harbored the qnrB, 1 (1.17%) of them contained the qnrS, 1 (1.17%) isolate carried all the three qnrA, qnrB, qnrS genes, 24 of 85 (28.2%) Salmonella carried blaCMY and 5 (5.88%) isolates carried qnrA and blaCMY. Antimicrobial susceptibility patterns of isolates were as a follow: 49 (57.6%) exhibited resistance to Nalidixic acid and none of them to Ciprofloxacin. 33 (38.82%) isolates exhibited resistance to Cephalosporins and 2 (2.35%) of them exhibited ESBL phenotype and 12(14.1%) isolates resistance to Ampicilin. MIC assay confirmed these results.

Conclusion: Having detected for qnr and bla genes suggested that these genes spread the resistance among diseases bacteria.

Keywords: QnrA, QnrB, QnrS, BlaCMY,Resistance Genes, Salmonella

P897: **Polymorphism analysis of Apical Membrane Antigen-1 coding gene in Iranian Plasmodium vivax isolates to evaluate it's potential as local MALARIA vaccine candidate**

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Background and Aim: Plasmodium vivax remains an important cause of morbidity outside Africa, and no effective vaccine is available against this parasite. Despite the current therapeutic approaches, vaccination seems crucial to achieve malaria elimination because of the emergence of drug resistance parasites. Therefore, genetic polymophism in protective antigens has posed a big challenge for the management of the elimination program in the endemic area. Present study was designed to evaluate the sequence polymorphisms of apical membrane antigen 1(PvAMA1) as an important vaccine candidate in natural isolates from main endemic areas in southeast of Iran in borderline with Pakistan and Afghanistan by using sequences analysis. To evaluate further its potentials as vaccine candidate, we studied its sequence heterogeneity in part of the world, where so far there is no such information.

Methods: A total of 99 blood samples were obtained on admission from patients diagnosed with vivax malaria at the Malaria Health Center of Chabahar Public Health Department in Sistan and Baluchistan province. All samples were diagnosed by light microscopic examination, then, 1 ml blood sample was collected after informed consent from all participants. The genomic DNA was extracted by phenol/phenol-chloroform and ethanol precipitation. Amplification of pvama-1 ectodomain region was performed in two separate reactions. To inspect SNPs (Single nucleotide polymorphisms) in 37 Pvama-1 isolates from Chabahar, DNA sequencing was used. Afterwards, the obtained nucleotide and amino acid sequences were aligned with the corresponding reference strain, Sal-1 (accession no. AF063138) to identify the polymorphic sites in ectodomains of pvama-1. Also, Nucleotide and amino acid sequences were compared with complete pvama-1 sequences available in GenBank database

Results: The pvama-1 gene was successfully amplified from genomic DNA purified from 99 isolates collected from patients infected with P. vivax. Amplification of the first (corresponding to 20–959 nucleotides) and the second (corresponding to 766–1542 nucleotides) fragments with194-bp overlapping of two fragments was performed in two PCR reactions. Randomly, 37 isolates were selected for further sequence analysis of entire pvama-I gene. The amino acid sequence data of pvama-1 in all 37 sequenced samples demonstrated nucleotide changes, leading to non-synonymous mutation at 11 positions compared to Sal-1 sequence (accession no. AF063138). Moreover, in comparison with the Sal-1 pvama-1 sequence, 29 different allelic forms were found among analyzed samples. Sequence characterization of PvAMA-1 isolates, particularly B cell epitopes, regions involving in RBC binding and intrinsically unstructured/disordered regions (IURs) that are important for design of vaccines, showed limited antigenic diversity in this gene.

Conclusion: Sequence data generated from this study showed that PvAMA-1 is a good candidate to be included in the design of an effective synthetic malaria vaccine against P.vivax infection. Having such information on local malaria parasites is an important factor and prerequisite in understanding the epidemiology of malaria as well as implications for the development of acquired immunity and for local anti-malarial vaccine research

Keywords: Plasmodium vivax, PvAMA-1,malaria

P898: **Multilocus sequence analysis of clinical isolates of Mycobacterium tuberculosis in northeast of IRAN**

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Background and Aim: Mycobacterium tuberculosis , is one of the most successful human pathogens, infecting nearly one third of the people all around the world . Identification of the relationships between different clinical for epidemiology study has great significance to public health, In many bacterial species multilocus sequence analysis (MLSA) were used as a powerful method for the classification and phylogenetic analysis of closely strains . In this study we used these DNA sequences and Analysis of partial gene sequences of rpsl, lipR, katG, fgd1 to generate a highly robust phylogeny of Mycobacterium tuberculosis and report on the evaluation of the MLSA for 20 Mycobacterium tuberculosis strains.

Methods: 20 strain isolates were selected from pulmonary tuberculosis patients during 2011 to 2012, Single Nucleotide Polymorphism were identified by aligning genome sequence and The discriminatory power of each locus was measured with Hunter- Gaston Index (HGI). Five genes were amplified and DNA Sequencing was performed . gene sequences of rpsl, lipR, katG, fgd1, mprA were Analysed and the phylogenetic three was constructed by MLST tools based on Neighbor-Joining method and For each gene measure of "genetic distance" between the sequences by Distance matrix.

Results: Among twenty patients, 12 patients were male and the rest were female, the mean age of them were 52. After the Data analysis of SNPs we found 31 variable sites among the 20 strain these polymorphisms comprised 6 non synonymous (ns) SNPs, 25 synonymous (s) SNPs. finally 9 STs identified in MLST tools software, among these isolates 6,8,9,10,15 in regarded to existing were similar to CCDC5180 and CCDC5079. SNPs can also be used to measure evolutionary distances between strains, the maximum evolutionary distance were obsereved between strain 9 with 1,2,3,5,7,11,1,19 and strain8 with 1,2,3,7,14.

Conclusion: Despite the importance of SNPs for our understanding of the diversity of MTpopulations, the research community currently lacks a comprehensive database about SNP data. In this study we found 31 variables SNP sites, Most of the SNPs in MT in coding regions of the genome. Computation of the evolutionary Distance matrix was matched with ST dendrogram that obtained from character-base phylogeny, in place where the two strain considered as a one ST the distance base of them was zero and it is indicating that there was a little polymorphism among them.

Keywords: Mycobacterium tuberculosis, genotyping, MLSA

P899: **In Vitro Antibacterial evaluation of aqueous and methanolic extracts of Synechoccocus sp. against a number of human pathogenic bacteria**

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Background and Aim: The study of cyanobacterial secondary metabolites has become a fascinating field of microbiology during the last few decades. These microorganisms are known to produce metabolites with diverse biological activities. The growing concern about antibiotic resistant in patients with bacterial infections has recently led to the development of natural antimicrobials to control pathogens bacteria. Bacteria related human infections are the most common. Cyanobacterial secondary metabolites can be a good candidate for inhibition of many n pathogenic bacteria. The general objective of this study was the test of antibacterial activity of aqueous and methanolic extract of cyanobacterium Synechoccocus sp. against a number of pathogenic bacteria.

Methods: In this experimental study, the cyanobacterium Synechoccocus sp. ISC 106 was obtained from the Algal culture collection of research institute of applied science, ACECR, Tehran, Iran. Disk diffusion method was used to study the effect of antimicrobial and broth microdilution method was used to determine the minimum inhibitory concentration (MIC).

Results: The results indicated that Methanolic extract of Synechoccocus sp. has no significant effect against tested bacteria. The results also clearly showed that aqueous extract of Synechoccocus sp. have significant antibacterial activity against most pathogenic bacteria; So that maximum antibacterial activity was against Staphylococcus aureus (PTCC 1112) which the average zone diameter around it was 26.33 Millimeters. Minimum inhibitory concentration of aqueous extract against most tested human pathogen bacteria was 125 mg/ml, whereas it was 250 mg/ml against Staphylococcus aureus, Enterococos fecalis, Shigella dysentery, Citrobacter sp. and standard strain Enterococcus faecalis (PTCC 1237).

Conclusion: It is concluded from this study that extracts of Synechoccocus sp. shown antimicrobial activity against the human pathogenic bacteria used in the present investigation and antibacterial activity of aqueous extract of Synechoccocus sp. was more than methanolic extract of Synechoccocus sp. . Improvement knowledge of the composition, analysis, and the properties of these cyanobacteria with respect to antimicrobial compounds would assist in efforts for the pharmaceutical application.

Keywords: Antibacterial activity, Broth microdilution, pathogen bacteria, Synechoccocus sp.

P900: **Antibacterial activity from methanolic extracts of cyanobacteria Synechoccocus sp. and Fischerella sp. strain against several species of Food-born pathogenic bacteria**

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Background and Aim: Natural products are an important source of new pharmaceuticals and pharmaceutical 'lead' compounds. Natural products have been isolated from a wide variety of taxa and tested for various biological activities. Cyanobacteria have been identified as a new and rich source of bioactive compounds. They are known to produce metabolites with diverse biological activities such as antibacterial, antifungal, antiviral, anticancer and algicide. The growing concern about food safety has recently led to the development of natural antimicrobials to control food-borne pathogens and spoilage bacteria. Bacteria related food poisoning are the most common, but fewer than 20 different bacteria actually are the culprits. Cyanobacterial secondary metabolites can be a good candidate for inhibition of many these bacteria. The aim of this study was test of antibacterial activity of methanolic extract of two cyanobacteria strains against seven species food-born pathogenic bacteria.

Methods: In this experimental study, two cyanobacteria species, Synechoccocus sp and Fischerella sp, was obtained from the Algal culture collection of research institute of applied science, ACECR, Tehran, Iran. The antibacterial activities of cyanobacterial extracts were assayed by agar disc diffusion method.

Results: Methanolic extracts from two strains of cyanobacteria were screened against seven strains of food-born pathogenic bacteria. Methanolic extract of Fischerella Sp. showed significant antibacterial activity but methanolic extract of Synechoccocus Sp no considerable effect against Staphylococcus epidermidis and Bacillus pumilus while strongly effective against Escherichia coli and Enterobacter aerogenes. The maximum effect of the methanolic extracts of Fischerella sp was seen against Bacillus cereus while the maximum effect of Synechoccocus sp. extract was seen against Escherichia coli. Results showed that antibacterial activity of Fischerella sp. was more than Synechoccocus sp. against seven species food-born pathogenic bacteria.

Conclusion: It is concluded from this study that extracts of Synechoccocus sp. and Fischerella sp. strain have significant antimicrobial activity against the food-born pathogens used in the present investigation.

Keywords: Antibacterial activity, Cyanobacteria, Food-born pathogen, Natural products

P901: **Antibiotic resistance to non- β- lactam antibiotics in ESBL producing and non-ESBL producing Pseudomonas aeruginosa bacteria**

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Background and Aim: Pseudomonas aeruginosa is an aerobic Gram negative bacterium which has emerged as one of the most problematic nosocomial pathogens. Concerned with the high rate isolation of P. aeruginosa among hospitalized patients in Mashhad, we decided to investigate about their antibiotic resistance pattern and comparison of resistance to non- β- lactam antibiotics in ESBL (extended spectrum β- lactamases antibiotic) producing and non- ESBL producing P. aeruginosa isolates.

Methods: Clinical isolates of P. aeruginosa were collected from different samples of hospitalized patients in Mashhad in the last five months of 2012. Bacteria were identified by usual differential biochemical tests. Antimicrobial assay of antibiotics was determined on Mueller- Hinton agar (Merck) using disc diffusion method. β- lactamase production was phenotypically characterized using double disk synergy test and confirmation disc method according to Clinical Laboratory Standards Institute.

Results: Out of 64 clinical isolated bacteria, 14 (21.9%) isolates were β- lactamase producers. Susceptibility of P. aeruginosa isolates to gentamicin, ciprofloxacin, amikacin and co- trimoxazole were respectively 43.8%, 53.1%, 64.1% and 0%. Resistance to gentamicin, ciprofloxacin, amikacin and co-trimoxazole for ESBL producers were 42.9%, 28.6%, 21.4%, 92.9%, and for non- ESBL producers were 50%, 44%, 38%, and 98%, respectively. There was no association between resistance to non- β- lactam antibiotics and ESBL producing among isolated bacteria. Conclusion: Among ESBL producers, the highest resistance was to cotrimoxazole and the lowest was to amikacin.

Conclusion: Due to the lack of significance correlation between resistance to examined antibiotics and production of β- lactamase, it is likely that genes encoding resistance to these antibiotics could not be co-transferred with ESBL genes.

Keywords: Antibiotic resistance, Extended spectrum β- lactamase, Non β - lactam antibiotics, P. aeruginosa

P902: **Colorimetric Detection of Antimicrobial Resistance pattern of Enterobacteriaceae**

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Background and Aim: Increasing antibiotic resistance of bacteria is a significant health, social and economic problem. The problem increases morbidity and mortality of humans. Enterobacteriaceae are a family of bacteria that commonly cause infections in health-care settings as well as in the community. Rapid detection of antimicrobial resistance of bacteria is beneficial for patients . In this study we have detected antimicrobial resistance patterns of clinical isolates of enterobacteriaceae by a rapid colorimetric method developed at Alzahra university.

Methods: One hundred Clinical isolates of enterobacteriaceae isolates were included in the study. Antimicrobial resistance patterns of all isolates were measured by the colorimetric method between 3.5-6 hours.

Results: Among a total of 100 isolates, resistance rates to cefazolin, cefotaxime, amikacin, imipenem,gentamycine,piperacilin , cotrimoxazole and ciprofloxacin were respectively 61%, 56%, 22%,17%, 36%,20%,57% and 31%.

Conclusion: The colorimetric method was assessed as a rapid method for detection of antimicrobial resistance patterns of enterobacteriaceae.

Keywords: Antibiotic, Enterpbacteriaceae ,Resistance

P903: **Prevalence of metallo-β-lactamase among imipenem-resistant Acinetobacter strain Isolated from hospitals patient from Khorramabad shohadaye Ashayer Hospital**

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Background and Aim: Background and aim: Acinetobacter are Gram-negative bacteria commonly involved in nosocomial infections.Imipenem-resistant Acinetobacter spp. resulting from metallo-β-lactamase (MBLs)-producing strains have been reported to be among important causes of nosocomial infections and of serious therapeutic problem worldwide.To determine the prevalence of metallo-β-lactamase among imipenem-resistant Acinetobacter isolates based on phenotypic methods.

Methods: Material and Methods: From November 1391 until June 1392, 34 isolates of Acinetobacterfrom select hospitals of patients in Khorram Abad ShohadayeAshayer hospital were identified with routine tests. Antibiotic susceptibility test by Disk - Diffusion method was performed according to CLSI.formetallo-β-lactamase production using combined disk diffusion, double disk synergy test.

Results: Of 34 isolates, 29(85.2%) were imipenem-resistance Acinetobacter .Positive phonetypictest for metallo-?-lactamase was 21(61.7).

Conclusion: conclusion: According to the presence of metallo -β lactamases in bacterial strain in the region.leading to difficulties in antibiotic therapy.

Keywords: Key words: metallo-β –lactamase,imipenem, Acinetobacter, nosocomial infections

P904: **Emergence of Imipenem Resistance and perevelance of Metallo B-Lactamases Enzymes in select clinicals Pseudomonas aeruginosa isolated from Khorram Abad Shohadaye Ashayer Hospital**

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Background and Aim: Background and aim: Pseudomonas aeruginosa is one of the opportunistic pathogenes in the hospital. It involves in burn , respiratory patients, people with genetic diseases of cystic fibrosis , bacterimia, septisemi and many of other prevelant infections.Pseudomonas aeruginosaisproblematic pathogens of clinical that usually by various mechanisms production of β -lactamase such as metallo-β-lactamase that can most of hydrolysis β-lactam antibiotics that shows resistance to varios antimicrobial agent

Methods: Material and Methods: From November 1391 until June 1392, 47isolatesPseudomonas aeruginosa of from select hospitals of patients in Khorram Abad ShohadayeAshayer hospital were identified with routine tests. Antibiotic susceptibility test by Disk - Diffusion method was performed according to CLSI.formetallo-β-lactamase production using combined disk diffusion, double disk synergy test (DDS)

Results: Result: Of the 47Pseudomonas aeruginosa isolates 24 (51.06%)were resistance to imipenem.Positive DDStest for metallo-?-lactamase was 19(40.4%)

Conclusion: conclusion: The result shows that the majority of isolated antibiotic resistance is an alarming,andtheir,s cure face whit ample problems

Keywords: Key words: metallo-β –lactamase,imipenem,Pseudomonas aeruginosa,imipenem

P905: **Identify species of dermatophytes using random primers OPD18 by AP-PCR method**

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Background and Aim: Dermatophytes are closely related keratinophilic fungi that invade the kratinolised tissues in humans causing dermatophytosis. Identification of dermatophytes with conventional methods is time consuming and sometimes problematic because of similarities and variabilities of species. Genetic amplification has made rapid and perfect identification of dermatophytes possible. The aim of this research is evaluation of AP-PCR method for identification of dermatophytes and to find a suitable approach for a rapid distinguish of dermatophytes. AP-PCR amplification is achieved through the use of short random primer (usually 10 nucleotides long) at relatively low temperature (normally <40 C).

Methods: Fifty-two isolates from 10 species of dermatophytes include: T. mentagrophytes (10), T. verrucosum (9), T. rubrum (5), T. tonsurans (3), T, violaceum (2), T. schoenleinii (1), M. gypseum (8), M. canis (4), M. ferrugineum (2), E. floccosum (8) were collected and confirmed with microscopic, macroscopic and biochemical tests. After extraction DNA, Molecular identification was done using AP-PCR (arbitrarily primed PCR) with random primer OPD18. These primers amplified bands of different sizes in species of dermatophytes DNA.

Results:: All species of dermatophytes were recognized with a distinct DNA band patterns on gel agarose. The range of obtained bands was between 250 to 1600 bp for all dermatophyte.

Conclusion: In laboratory always have problems on recognizing T. mentagrophytes from T. rubrum but using this method, recognizing two species of fungus can easily done. AP-PCR is a very sensitive method, so that the slightest change in the concentration of DNA, primer, MgCl2 may get different results using this approach is not recommended as a diagnostic procedure.

Keywords: Dermatophyte, random primer OPD18, AP-PCR

P906: **Rapid and specific detection Shiglella flexneri by PCR using specific virulence factor gene**

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Background and Aim: Shiglella is an intestinal Gram-negative bacilli whose infections has created many serious problems in developed and developing countries. The outbreak of Shigella is a very easy procedure due to its low pathological and easy transition from a person to another one, indeed people`s indirect infection via food and contaminated water is very easy. IpaH is one of those gens that can be used as reagent to diagnose. The purpose of this study is to use PCR method as a quick diagnosis of Shigella with high sensitivity

Methods: In the study, Standard bacterial strain S. flexneri used for optimization the test. The genomic DNA was extracted from broth cultures of S.fellexneri by gram negative kit. Subsequently, a pair of particular primer was used to duplicate IpaH gen through PCR to identify Shigella type.

Results: The result of this study showed that the standard strain produce target fragment. In fact designed primers produce a 169 bp fragment.

Conclusion: due to IpaH gen existence in Shigella type, the method can be used as high sensitive with less time consuming and as practical one to identify Shigella flexneri and other types in disease samples

Keywords: Shiglella flexneri ,polymerase chain reaction , IpaH gen

P907: **Prevalence of Class I Integrons among Pseudomonas aeruginosa Isolates from Patients with cystic fibrosis and Burn infections**

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Background and Aim: Pseudomonas aeruginosa is a ubiquitous organism that can cause disease in wide range of beings and found in soil, water, skin flora, and most environments frequently. In other hand the bacterium as opportunistic pathogen in the nosocomial setting and immune compromise patients is highly regarded. The intrinsic antibiotic resistance in addition to distinctive capacity of Pseudomonas aeruginosa to acquire multiple resistance mechanisms to all the antibiotics available commercially is notable and alarming. Integrons as genetic elements have distinctive role in the distribution of multidrug resistance feature among gram negative bacteria in particular in Pseudomonas aeruginosa. The class 1integron as a primary source of resistance genes can quickly distribute antibiotic resistance genes between gram-negative and - positive bacteria and led to increasing problem in infectious disease. The aim of current study was to investigate prevalence of Class I Integrons among Pseudomonas aeruginosa Isolates from Patients with cystic fibrosis and Burn infectionsin Tehran, Iran.

Methods: Throughout April 2012 to July 2012, 77 isolate of pseudomonas aeruginosa were obtained from patient with burn infection who refer to Motahari hospital, and also patients with cystic fibrosis referring to the Mofid Educational Hospital Tehran, Iran. All isolates were identified by routine phenotypic and biochemical tests. Template DNA was extracted by using boiling method and then PCR was pre¬pared. All extracted DNA were screened for integron class 1 cassette by specific designed prime. Results were visualized by ethidium bromide staining under UV transluminator.

Results: From 33 samples isolated from burn patient who screened for the presence of class 1 integron twenty –one isolates (81.81%) were identified as being positive for class 1 integron and 18.18% were negative. From 44 samples isolated from cystic fibrosis patient fourteen (31.81%) were positive and thirty samples were negative against class 1 integron. In general from 77 collected samples forty -one (53.24%) samples were positive and thirty –six (46.75%) reported as negative result.

Conclusion: Resistance was common among most Pseudomonas aeruginosa isolates from different sources. There was a significant differences in integron class 1 distribution among isolates from burn and cystic fibrosis ($p<0.05$) shows burn isolates are more likely nosocomial and more resistant. There have been some reports that describing the prevalence of class 1 integrons within Gram-negative clinical isolates. Studies of selected clinical bacterial populations have demonstrated 59-75% of antibiotic-resistant isolates contain class 1 integrons which is in accordance with our study. Study of other resistant mechanisms is recommended.

Keywords: Pseudomonas aeruginosa ,Class I Integron,Burn infection,cystic fibrosis

P908: **Evaluation of Synthetic 4-hydroxy Coumarin derivatives efficacy against most important Gram-positive organisms**

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Background and Aim: Today, our armory against infection is continually being depleted, as small enemies are crafty warriors that develop resistance to current antibiotics. Improper use of antibiotics especially in developing countries has led to the emergence of multi and pan drug resistant bacterial strains. The Spread of superbugs like methicillin-resistant Staphylococcus aureus (MRSA), Vancomycin-resistant Enterococcus faecium (VRE) to the other parts of the world are considered a global health concern. Need for discovery and Synthesis of new antibacterial compounds is urgent. 4-hydroxyCoumarin derivatives because of their pharmaceutical activities are highly regarded so Coumarin-containing antibiotics, Novobiocin, coumermycin, and clorobiocin can be synthesized by number of microorganisms. tendency of some bacteria, to develop resistant to coumarins based antibiotic during therapy as well as the Novobiocin toxicity towards eukaryotic cells, efforts to discovery and optimization of novel and potential DNA gyrase inhibitors to be continued. we attempt to synthesize of bis(4-hydroxycoumarin) derivatives in a simple experimental procedure by thiourea or urea as a green catalyst .antibacterial activity of synthetic compounds by well diffusion and broth micro dilution methods was evaluated.

Methods: A mixture of 4-hydroxycoumarin (2 mmol), an aromatic aldehyde (1 mmol) and thiourea or urea (1.3 mol %) in EtOH (3 ml) for the synthesis of bis(4-hydroxycoumarin) derivatives were stirred at 80 °C for the six hours. Upon completion of the reaction, the products were monitored by TLC and reaction mixture was allowed to cool to room temperature and the solid phase was filtered off. Then the structures of compounds were confirmed by IR, 1H NMR, 13C NMR and MS spectrograms. The Gram posetive bacterial model were Staphylococcus aureus ATCC 25952, Staphylococcus aureus ATCC 25923, Staphylococcus aureus ATCC 29213, Enterococcus faecalis ATCC 29212, Enterococcus faecalis ATCC 33186, Listeria monocytogenes ATCC 7644, clinical isolate of Streptococcus pyogenes, Streptococcus salivarius PTCC 1448, clinical isolate of Staphylococcus saprophytius, Streptococcus mutans PTCC 1683, Streptococcus sanguis PTCC 1449, Bacillus subtilis ATCC 6633 and gram negative selected models were Pseudomonas aeruginosa ATCC 27853 and Escherichia coli ATCC 25922 While in the broth micro dilution method we used only the gram posetive bacterial models. A 0.5 McFarland concenteration of each models inoculated to The Mueller-Hinton agar (Merck) plates. A stock solution of synthetic compounds was prepared 0.01 gram of each component was dissolved in 1 ml dimethyl sulfoxide (DMSO). A concentration of 1 mg/ml or 100 µg/0.1ml of each sample was transferred into each respective labeled well. After 24 hour inhibitory zone of each sample was measured. MIC broth micro dilution method performed according CLSI reference.

Results: In well diffusion and broth micro dilution survey, most synthesized compounds revealed acceptable antibacterial activity against gram positive bacterial models .

Conclusion: Compounds 1a, 2a 3a, 4a, 6a, 7a and 8a shown admisible anti gram psetive bacterial activity. Compound 1a with phenyl group, 3a as new compound and 4a with bromine bulky and electrophilic functional group, 7a with methyl group and finally 8a with phenyl chloride group shown better anti gram posetive activity in MIC test.

Keywords: Synthetic 4-hydroxycoumarin,anti bacterial activity,DNA gyrase inhibitor

P909: **Study of the aminoglycoside resistance genes acc(3)-Ia, aac(6')-Ib, acc(3)-IIa و, armA and rmtB in Enterococcus isolates**

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Background and Aim: Aminoglycosides are the most commonly used antibiotics in the treatment of infectious by both Gram-negative and Gram-positive bacteria. They bind to the ribosomes and thus interfere with protein synthesis. The major mechanism of resistance to aminoglycoside antibiotics in clinical bacterial isolates is the production of three types of aminoglycoside-modifying enzymes, aminoglycoside phosphotransferases (APHs), aminoglycoside acetyltransferases (AACs), and aminoglycoside nucleotidyltransferases (ANTs). The 16S rRNA methylase confers high-level resistance to 4,6-substituted deoxystreptamines, including arbekacin, amikacin, kanamycin, tobramycin and gentamicin, by posttranscriptional methylation of 16S rRNA leading to loss of affinity for aminoglycosides. The purpose of the present study is to investigate the prevalence of aminoglycoside resistance genes aac(6')-Ib, acc(3)-IIa, acc(3)-Ia , armA and rmtB Enterococcus isolates.

Methods: 117 clinical strain's of Enterococcus isolated and Antibiotic susceptibility for screening of resistance isolates was done by Kirby-Bauer method for Gentamicin, Amikacin, Kanamycin and Tobramycin. The acc(3)-Ia, aac(6')-Ib, acc(3)-IIa and 16SrRNA methylase genes(armA and rmtB) were detected by PCR amplification

Results: Among isolates, the most resistance observed for kanamycin(68/4%) and Amikacin (68/4%). Resistance for Tobramycin and Gentamicin was 42.7% and 28.2% respectively. 93 isolates of Enterococcus were resistance at least for one of aminoglycosides. Among Enterococcus isolates with resistance susceptibility, 72.04% , 66.7% and 36.6% isolates shown the acc(3)-Ia, acc(3)-IIa and aac(6')-Ib respectively and armA and rmtB observed in non of isolates

Conclusion: The level of resistance to aminoglycoside antibiotics is influenced by many factors, including the amount of modifying enzyme produced and the rate of penetration of the antibiotic into the bacterial cell and efflux pumps. These results indicate the high prevalence of aminoglycoside antibiotics resistance in Enterococcus isolates.The acetyltransferase modifying enzymes have the main role in this resistance and there is a significant relationship between the incidence of aminoglycoside resistance and presence of aminoglycoside resistance genes

Keywords: Enterococcus, aminoglycoside resistance genes, 16S rRNA methylase

P910: **Survey The prevalence of TEM genes and antimicrobial resistance among the Escherichia coli and Klebsiella pneumonia in patients with nosocomial infections in Shahid beheshti university hospitals in**

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Background and Aim: Extended spectrum β-lactamases(ESBLs) have emerged as a major threat worldwide with limited treatment options. This study aimed to determine the antibiotic susceptibility pattern and the evaluation of TEM genes producing in Escherichia coli and Klebsiella pneumonia isolates collected from clinical samples.

Methods: Bacteria were isolated and identified from the different samples in patients sent to laboratory of shahid beheshti university in Tehran in 2011. Isolates were then tested for antimicrobial susceptibility by disc diffusion and examined for TEM genes production by polymerase chain reaction using specific primer.

Results: Out of 100 studied nosocomial infection specimens, 50 isolates were klebsiella pneumonia of which 34 percent were ESBL producer and all were positive for TEM gene, resistance of Cefotaxime was 90 percent which is the highest degrees of resistance and lowest degrees of resistance to Imipenem was 4 percent.Among the 50 isolates of Escherichia coli of which 14 percent were ESBL producer and all were positive for TEM gene, resistance of Cefexime was 90 percent which is the highest degrees of resistance and lowest degrees of resistance to Meropenem was 6 percent.

Conclusion: Due to relatively high prevalence of ESBL-producing bacteria in the studied population , antibiogram test are advised for appropriate treatment.

Keywords: Antibiotic sensitivity, Extended spectrum beta-lactamase, Klebsiella pneumoniae

P911: **Determination of nucleotide sequence of conserved region of ompP5 in clinical isolates of Haemophilus influenzae spp with the aim of vaccine design**

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Background and Aim: Haemophilus influenzae strains are divided according to capsular polysaccharide to typeable and nontypeable (NTHi). Available H. influenzae type b (Hib) vaccines consist of polyribosyl ribitol phosphate (PRP) coupled to a protein carrier and does not develop active immunity against infections caused by H. influenzae in children aged 3 month until 3 years. Protein 5 is one of the H. influenzae Outer membrane proteins (OMPs) that expressed by Hib and NTHi. The purpose of this research is determination of nucleotide sequence of variable region of the gene encodinig P5 (ompP5) in clinical isolates of H. influenzae spp isolates for further vaccine studies.

Methods: Clinical samples from 21 out patient of Imam Khomeini and Milad hospitals suffering from pneumonia and meningitis are collected. ompP5 amplified using primers that designed for conserved region of this gene. By detecting the subsequence and comparing the subsequences of ompP5 gene for 5 clinical strains of H. influenzae with standard strain, the similarities and differences between them were found.

Results: Variable region of ompP5 was amplified in 17 samples. Results of subsequence detection for the variable region of ompP5 for 5 samples was 96.88%, 100%, 99.22%, 99.02% and 96.88% similarity with conserved region of ompP5 of Hib

Conclusion: ompP5 in both strains of Hib and NTHi is highly conserved and thus could be used as a vaccine candidate to against all of H. influenzae infections after further investigation.

Keywords: Haemophilus influenzae, ompP5, Nucleotide sequencing

P912: **Evaluation of biofilm-forming capabilities of urinary Escherichia coli isolates in microtiter plate with using two different culture medium**

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Background and Aim: The ability of uropathogenic E. coli (UPEC) to cause symptomatic UTI is associated with the expression of a variety of virulence factors, biofilm formation is one of these factors. Biofilm formation makes bacteria resistant to the flow of urine and has increased tolerance to antimicrobials and the host immune response. Types of culture media affect the biofilm-forming capabilities. The aim of this study was to evaluate biofilm-forming capabilities of urinary Escherichia coli isolates in microtiter plate with using two different culture media.

Methods: A total of 170 isolates of E. coli were collected from patients having symptoms of UTIs in Gorgan - north of Iran. Biofilm formation based on the method proposed by Watnick and Meshram in LB medium and BHI broth with the addition of 1% sucrose were investigated. The quantitative analysis of biofilm formation was performed by staining crystal violet and after adding decolonization solution was measured by spectrophotometry(ELISA reader).

Results: The biofilm formation of UPEC strains in LB medium and BHI were 20 (11.8%) and 105 (61.8%) isolates, respectively, that this difference is statistically significant ($P<0.001$). All isolates that were formed biofilm in LB medium can also form biofilm in BHI medium. While 36 (21.2%) isolates in BHI formed strong biofilm , Only one(0.6%) isolate in LB medium can too , that this difference is too statistically significant ($P<0.007$) .

Conclusion: our finding demonstrated that the biofilm formation of UPEC in BHI broth with the addition of 1% sucrose was better than LB medium. To find the best conditions of biofilm formation, change on culture media and incubation conditions were suggested.

Keywords: Biofilm, uropathogenic E. coli (UPEC), LB medium, BHI medium

P913: **Comparison of formaldehyde and thiomerosal in detoxification of whole cell pertussis vaccine**

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Background and Aim: Pertussis (whooping cough) is a bacterial respiratory infection caused by Bordetella pertussis, a gram negative bacillus. Production of pertussis vaccine started in Razi Institute since 1949 using Bordet- Gengou agar. Heat inactivation following 6-9 months and thiomerosal detoxification at 4-8°C had been also used for downstream processing of the vaccine. Since detoxification of B. pertussis toxic components by thiomerosal at 4-8°C is a long term procedure which is required more cooling spaces to store bacterial suspensions and also there are some reports on weakness of this method for removal of pertussis toxic activities. In this project, we aimed to use formaldehyde and thiomerosal as two common detoxifier agents for detoxification of pertussis cell suspensions and compare their effects on the products in terms of toxicity and efficacy.

Methods: Six batches of B.pertusis EXMG89-1, EXB89-16, EXB89-14, EXB89-13, EXB89-7 and EXB89-3 of strains 134 and EXMG89-2, EXB89-5, EXB89-17, EXB89-12, EXB89-11 and EXB89-6 of strain 509 were prepared and used to do the comparison. Two physical methods using centrifuge and microfiltration were used for separation of the bacterial cells from culture medium. The separated bacterial cells were resuspended with cold and sterile PBS in a concentration of 100-150 Iou/ml then were divided in to parts named formaldehyde detoxified (FD) and thiomerosal detoxified (TD) suspensions. FD suspensions were inactivated at 56ºC for ten minute with addition of 10 mM formaldehyde solution 37.7% and TD suspensions were inactivated at the same conditions with addition of 0.01% w/v thiomerosal solution. The two suspensions were incubated at 4-8 ºC for detoxification process. Samples were taken on day 10, 30, 90, 180 and 270 for mouse weight gain test to evaluate removal of pertussis toxicity. Finally six FD and TD experimental vaccines were prepared by mixing of equal cell concentrations of both strains 134 and 509. The prepared vaccines were then evaluated for their efficacy using mouse protection test (Kendrick test).

Results: The results indicated that formaldehyde method with a mean detoxification period of 25.4 days compared with 195 days period of thiomerosal method, significantly decreased the period of detoxification. The efficacy of fomaldehyde detoxified vaccines with a mean value of 5.9 was slightly less than of that obtained from thiomerosal detoxified vaccine with a mean value of 5.95.

Conclusion: Based on the results, it can be concluded that formaldehyde is more suitable than thiomerosal to be used for detoxification of B. pertussis suspensions with almost 7.5 times reduction in detoxification period at 4-8°C. While the mean potency value of thiomerosal detoxified vaccines was 0.05 unite higher than formaldehyde detoxified vaccines, their potency values showed no statistically significant difference (P 0.005).

Keywords: Bordetella pertussis; Toxicity; Formaldehyde detoxified; Thiomerosal detoxified

P914: **TEM and SHV Beta-Lactamases among Enterobacter Isolates Obtained from Tehran and Qazvin hospitals, Iran.**

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Background and Aim: Enterobacter spp. are important opportunistic pathogens known to cause nosocomial infections. Resistance to the extended-spectrum cephalosporins among these organisms has become a growing worldwide problem. The most clinically important enzymes in this group are TEM and SHV plasmid-mediated beta-lactamases. The aim of this study is to evaluate the prevalence of Extended-Spectrum Beta-Lactamases (ESBLs) in Enterobacter spp. clinical isolates by phenotypic and genotypic methods.

Methods: 120 non-duplicate Enterobacter isolates were collected from clinical samples from the patients admitted in Tehran and Qazvin hospitals, Iran. Combined Disk (CD) method were performed to detect the beta-lactamase enzyme. The ESBL types were determined by PCR using specific primers for blaTEM and blaSHV .

Results: The prevalence of ESBLs was 54/120 (45%) with 30 (25%) and 4 (3.3%) isolates testing positive for genes encoding TEM and SHV respectively.

Conclusion: The ESBL prevalence in Enterobacter spp. increased because of the wide use of the extended spectrum cephalosporins in clinical settings. Thus, the challenge to recognize susceptibility patterns indicative of the presence of specific b-lactamases will become even more important as the members of this genus acquire additional antimicrobial resistance in the future.

Keywords: Enterobacter spp., Extended-Spectrum Beta-Lactamase, Combination Disk, PCR

P915: **Retrieval and Quantitation of Human Papillomavirus (HPV) DNA in the Iranian of Patients with HPV-associated Retinoblastoma.**

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Background and Aim: Propose: Study and quantitative real-time PCR is a more sensitive and particular assay to monitor serum cell-free HPV DNA. Introduction: The human papillomavirus (HPV) has been implicated as an etiological factor in a subset of retinoblastoma. Because circulating tumor DNA has previously been detected in the Iran of patients with advanced retinoblastoma. we hypothesized that HPV DNA might be present in the Iran of HPV-positive retinoblastoma patients.

Methods: Methods: Serum DNA extracts from 70 patients with retinoblastoma were screened for HPV using conventional PCR and a real-time quantitative evaluation. All samples subjected to conventional PCR were further tested by dot blot hybridization, and positives were justified by Southern blotting.

Results: Results: Paired tumor DNA from recorded tissues was then alikely screened for HPV genomic material (n 5 51) or tested by in situ hybridization (n 5 19). HPV-16 DNA was detected with L1 primers in 0 of 65 Iran and in 15 of 70 (21%) tumors. Conventional PCR with E7 primers and Southern blot hybridization discover HPV-16 DNA in four (6%) serum. Using real-time quantitative PCR, six samples were establish to entail diverse levels of circulating HPV DNA (medial, 12 copies/ml; range, <1–35.) All six serum-positive patients had corresponding tumors positive for E7. However a much larger prospective test is needed, the attendance of HPV genomic material in serum DNA of HPV-positive retinoblastoma patients may serve as a beneficial marker of early metastatic disease.

Conclusion: conclusion: In this investigate, we report the retrieval of HPV DNA in the Iran of patients with HPV-associated retinoblastoma.

Keywords: Keywords: Genotyping, human papillomavirus, retinoblastoma. real-time PCR.

P916: **Retrieval of oncogenic human papillomavirus in sporadic retinoblastoma Iranian chilldren.**

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Background and Aim: Purpose: To investigate the attendance of human papillomavirus (HPV) DNA in tumour tissue from patients with unilateral retinoblastoma.Background: Retinoblastomas befall as the consequence of inactivation of the tumor prohibitive retinoblastoma protein (pRb), classically upon biallelic inactivation of the RB1 gene locus. Recently, human papillomavirus (HPV) genomic DNA has been discovered in retinoblastomas. The oncogenic potential of these viral proteins is specified by their authority to bind cellular proteins such as pRb and p53, thus interfering with their functions in the cell cycle. there is voucher that propose that HPV infection play a role in the sequence of occurrence that leads to the outspread of retinoblastomas, the implications of the viral attendance in tumour tissue and in normal retina must be confirmed by future studies.

Methods: Methods: Samples of paraffin-embedded tumour tissue from 43 baby with unilateral retinoblastoma were collected to investigate the presence of HPV DNA using polymerase chain reaction (PCR) and dot blot hybridization.

Results: Results: Oncogenic HPV DNA types 16 and 35 were detected in 12 (27.9%) of 43 tumour specimens. A higher frequency of differentiated tumours (63.3%) was observed among the HPV-positive tumours.

Conclusion: Conclusions: We research demonstrating the attendance of the papillomavirus genome in tumour tissue from baby with sporadic retinoblastoma propose that this virus has a conceivable role in the outspread of these ocular tumours. Future studies are needful to demonstrate an relationship between HPV and sporadic retinoblastoma.

Keywords: Key words: human papillomavirus; retinoblastoma; virus.

P917: **In silico study, cloning and expression of truncated forms of flagellin (fliC) fused to FimH from uropathogenic Escherichia coli**

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Background and Aim: Entroaggregative Escherichia coli (EAEC) is major cause of acute and chronic diarrhea worldwide. Flagellin (FliC) is a major bacterial surface protein of EAEC and structural component of flagella, locomotive organelles in wide range of bacteria. In addition to the role of flagellin in bacterial motility studies have shown that bacterial flagellin is able to activate innate immune responses via activation of Toll-like receptor 5 (TLR-5). We have previously shown that two truncated forms of FliC-EAEC containing the conserved N and C terminal portions are able to activate TLR-5. Based on the ability of truncated forms of FliC-EAEC to activate innate immunity, these forms could be considered as potent adjuvants in designing new vaccines. In this regard, this work focused upon in silico study, cloning and expression of fusion proteins consisting of truncated forms of FliC and uropathogenic Escherichia coli FimH as a recombinant vaccine against urinary tract infections.

Methods: FliC /FimH fusion proteins were modeled using I-Tasser server. The validity of structures was investigated using RAMPAGE and ProSA web. Modeled structures were docked to TLR-5 using Hex docking server. Depending on the energy value and pose of interaction best fusions were selected and subjected to cloning in to pGEX-5X-1 plasmid and expression in Top10 strain of E. coli.

Results: Fusion proteins consisting of truncated forms of FliC (Trunc A and B) at N- and C-termini (TruncA-FimH-TruncB) and C-terminal (FimH-TruncA-TruncB)of FimH showed the best interaction affinity to the receptor. Cloning of these fusions was confirmed by DNA sequencing and their expression was observed on SDS-PAGE and confirmed by western blotting.

Conclusion: According to our in silico results, two fusion proteins showed the best interaction tendency to the receptor. These fusions were successfully cloned and expressed in Ecoli system. Further in vitro and in vivo studies are still necessary to evaluate the ability of these constructs in activating immune responses.

Keywords: In silico study, FliC, FimH, Uropathogenic Escherichia coli

P918: **Studying and Comparison the Knowledge Rate of Urban and Rural Societies from Probiotic Products and its effect on Human Health at ahar City (Iran)**

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Background and Aim: Long years, Bacteria were known as human enemies; Perhaps it was strange But in fact, All Bacteria are not bad. Bacteria such as Listeria and Clostridium which each was causing to terrible disease, but many others from Bacterial family, not only have not any harm, but also are sometimes useful; And nowadays, at producing the Drug, Hormone , Vaccine , Enzyme and else, the microorganisms are used as a main component at production process. Meanwhile, probiotic Bacteria with ability to change Flora Microbial of Intestine, it is playing basic role as a useful Bactria at body.

Methods: Present study was a societal-sectional that is formed with use of questionnaire, and By going To people house of research and face to face interviewing and completing the questionnaire. Samples Were selected by random selection method. In this purpose, in base of the census in 2006, by systematic Selection method, 400 family and 20 classes of university was selected and this classes and families were a start point of each. Obtained information was analyzed by SPSS Software .: Results from people knowledge rate about probiotic products and its effect on human health and also at two groups in view of urbanite and rural settlement and also from view of education rate compared together and analyzed and needed results were obtained. According to the Information, 5 percent of people at Iran are stricken with bacterial diseases, that the rate of disease in both as sexes has been reported equally.

Results: Rate of bacterial diseases in this region was 43 in thousand, while in other regions of countries that was 25 in thousand, and many of bacterial disease can be decreased by giving Information and different training and also special consultation. This study showed that about 70% of people information of this area, about Bacterial diseases and its relationship with health system, is medium and less; and about 15% have introduction; and about 5% have no introduction about it. And also in this study, between two groups of urbanite and rural settlement and also persons with different education rate, significant difference (> p0.007) were observed. Regarded to recent results in the current society, It is seen that the primary information rate of people about the relationship between health and probiotic products, and its effect on human health, and the effect of probiotics in prevention and treatment and reducing diseases, and also increasing the society health, is less from average; And from view of existence of facilities and laboratories, approximately in 50-60% of society, there is no facilities and laboratories; And people are deprived.

Conclusion: people didn't know any society expert at this course and have a little information about it. In this conditions, offering effective strategies and creating an appropriate conditions to promoting awareness level, and interesting the society from antibiotic products, is necessary.

Keywords: Probiotic, Pathogenic bacteria, Human Health

P919: **In vitro activity of Quercus brantii extracts against biofilm – producing Pseudomonas aeruginosa**

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Background and Aim: Bacterial adhesion and biofilm formation by Pseudomonas aeruginosa is a serious concern in the medical industries. Biofilms have also been implicated as the cause of serious infections in humans as their occurrence makes it difficult to treat common infections and the likelihood of recurrent infections is high. Due to emerging resistance, conventional control methods are fast becoming ineffective.Natural products that originate in plants can influence microbial biofilm formation. The effect of ethyl acetatic, methanolic and water- methanolic extracts of Quercus brantii on biofilm formation and biofilm disruption of clinical and reference (ATCC 27853) strains of P. aeruginosa were investigated in this study.

Methods: The inhibitory activity of Q. brantii extacts (in 1/2 MIC , MIC and 2 MIC concentrations) on P. aeruginosa biofilms was tested on polystyrene flat – bottomed microtitre plates using the crystal violet (CV) staining assay. The CV assay was used to assess the effect of pre-treating a surface with plant extracts on cell attachment and the extent of biofilm development (biofilm biomass). Disruption of pre-formed biofilms were also investigated following exposure to extracts in different concentrations (0.025- 0.4 g/ml)

Results: The results showed that inhibitory activity was dependent on the extracts concentration . Most of the extracts reduced microbial colonization by at least 50%. Ethyl acetatic extract of Q. brantii , was the most effective extract and inhibited P. aeruginosa biofilms (clinical strains and ATCC 27853) over 70% in most of the concentrations . It is followed by water- methanolic extract (52% - 66% inhibition) and methanolic extract (44% - 57% inhibition) of Q. brantii. Disruption of pre-formed biofilm of ATCC strain needed to high concentration (0.4 g/ml) of all the extracts. But biofilms of clinical strains were more sensitive and disrupted at lower concentrations (0.05- 0.2 g/ml).

Conclusion: This study demonstrated the anti-adherence and antibiofilm activity of Quercus brantii extracts and points out the exceptional efficiency of them , which could represent candidates in the treatment of P. aeruginosa biofilm. Also our findings showed that there was a relationship between biofilm strongness and concentration of plant extracts for disrupting biofilms.

Keywords: biofilm, adherence, plant extracts, Pseudomonas aeruginosa

P920: **A antimicrobial activity of forty- five medicinal plant extracts against Pseudomomas aeruginosa planktonic cells**

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Background and Aim: Pseudomonas aeruginosa is one of the common pathogenic causes of serious infections in burn patients throughout the world. Resistance against many antibiotics in this pathogen is accumulating. Therefore, searches for new substances with antimicrobial activity have become an urgent necessity. Medicinal plants are frequently used in popular medicine as remedies for many infectious diseases. This study aimed to determine the in vitro antimicrobial activity of ethyl acetatic , methanolic and water- methanolic extracts from different parts (root, aerial parts including leaf and stem , fruit, seed) of fifteen different plant species against P.aeruginosa (ATCC 27853)

Methods: Forty - five extracts from all the following plants: Satureja sahendica (root and aerial parts), Hymenocrater bituminosus (aerial parts), Salvia hypoleuca (root and aerial parts), Salvia macrosiphon (aerial parts), Scutellaria tournefortii (aerial parts), Perovskia artemisoides (aerial parts), Cymbocarpum erythraeum (aerial parts), Ferulla oopoda (aerial parts), Teucrium polium (aerial parts), Peganum harmala (seed), Quercus brantii (fruit), Medicago sativa (aerial parts), Satureja mutica (root), Ferula pseudalliacea (aerial parts) and Satereja khuzestanca (aerial parts) were screened for their antimicrobial activity , using agar disc- diffusion and microbroth dilution assays.

Results: Results demonstrated that these extracts had inhibitory effects at different concentrations (0.05, 0.1, 0.2 and 0.4 g/ml) against P.aeruginosa (ATCC 27853). The highest inhibitory zones were showed by ethyl acetatic extract of Satureja sahendica (20.33 ± 0.47mm). It is followed by methanolic extract of Salvia hypoleuca (17 ± 0.81 mm) ,water-methanolic extract of Satureja sahendica (16.66 ± 0.47 mm) and methanolic extract of Hymenocrater bituminosus (15.33 ± 0.47 mm). Quercus brantii showed good antibacterial property in all of extracts including ethyl acetatic , methanolic and water- methanolic extracts with inhibition zones of 18 ± 0.81mm ,14.33 ± 0.47mm and 14.66 ± 0.47mm ,respectively. Also all of the extracts from Quercus brantii were the most active against P.aeruginosa with minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of 3.125 and 12.5 mg/ml for methanolic and water- methanolic extacts and 6.25 and 25 mg/ml for ethyl acetatic extract,respectively. It is followed by methanolic extract of Ferula pseudalliacea (MIC = MBC= 12.5 mg/ml) and ethyl acetatic extract of Satureja sahendica (MIC= MBC = 50 mg/ml).

Conclusion: Our results revealed that , among the 15 tested plant species , Quercus brantii, in general , had the most antibacterial effect against P.aeruginosa (ATCC 27853) .

Keywords: plant extracts, antibacterial activity, Pseudomonas aeruginosa

P921: **Preparation LPS of Pseudomonas aeruginosa as an Antigen to Evaluate the Immunogenicity**

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Background and Aim: Pseudomonas aeruginosa is one of the most important agents of infections specially in immune suppressed, cancer, Cystic fibrosis and severe burns. The main purpose of this study is providing extracted (LPS)Lipopolysaccharide as an antigen to produce antibody and vaccine. LPS is the most important antigen structure.

Methods: Firstly, this bacterium was cultured massively and its biomass was centrifuged. LPS was extracted and concentrated by cold ethanol. Acetic acid was added in high temperature to depolymerization of LPS. The solution was dialyzed and qualities controls tests were done.

Results: The results indicated that the extracted LPS was in acceptable range. The sterility, nucleic acid measurement, protein measurement and pyrogenic toxicity tests showed that the extracted LPS is suitable for vaccines goals.

Conclusion: Qualities controls tests certificated for extracted LPS and it can be used for study as a vaccine.

Keywords: Pseudomonas aeruginosa, LPS, Vaccine, Extraction.

P922: **Comparison the Immunogenicity of Pseudomonas aeruginosa D-LPS-Exotoxin A conjugate and D-LPS against P. aeruginosa Infections**

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Background and Aim: According to antibiotic resistanc , appropriate drugs controlling P.aeruginosa infection are currently limited and Pseudomonas infections are difficult to treat . P.aeruginosa Lipopolysaccharide is a key factor in virulence. It has been shown that Antibody to LPS is highly protective against P.aeruginosa infections in a variety of animal models. Conjugation of Lipopolysaccharide antigens to carrier proteins currently used to enhance their immunogenicity. This converts Lipopolysaccharide from a T-cell-independent to a T-cell-dependent antigen, and elicits a higher and booster immune response in animals.

Methods: LPS from P.aeruginosa was extracted with modified hot phenol method and detoxified by NaOH. For conjugation to recombinant Exotoxin A(rEPA), ADH was linked as a spacer molecule and EDAC as a linker. D-LPS-Exotoxin A was purified by gel filtration. D-LPS-Exotoxin A and D-LPS were injected to animal model intra peritoneally. Vaccination was performed by 3 doses.Then serum samples were collected and antibodies response against LPS was measured by ELISA method for total IgG,IgM,IgA,IgG1,IgG2a,IgG2b and IgG3.

Results: The results of anti-LPS inductions for total IgG,IgM,IgA,IgG1,IgG2a,IgG2b and IgG3 were observed D-LPS-Exotoxin. These results indicated that LPS from P.aeruginosa increased anti-LPS antibodies in conjugated with Exotoxin A and can be appropriate effective conjugate .

Conclusion: D-LPS-Exotoxin A showed consequential increasing in all types of antibodies titers concentration against LPS in versus D-LPS ,accordingly conjugate from P.aeruginosa will be useful tools for elucidating pseudomonas infections.

Keywords: P. aeruginosa, conjugate, D-LPS,Recombinant, D-LPS-Exotoxin A, Immunogenicity

P923: **Bacterial agents and their antibiotic susceptibility profiles of neonatal septicemia**

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Background and Aim: Neonatal septicemia is an important cause of morbidity and mortality. Since etiological agents of neonatal septicemia and their antimicrobial susceptibility pattern may differentfrom one region to another, knowledge of the prevalence of the local isolates and their antimicrobial sensitivity pattern is of utmost importance for prompt institution of antimicrobial therapy. The purpose of the present study was to identify the common agents of neonatal septicemia and to determine their antimicrobial susceptibility pattern.

Methods: This was a prospective cross- sectional study performed on a total of 491 samples from NICU of Namazi Hospital in Shiraz, Iran during 21 months period since February, 2012.Specimens were collected from the neonates with clinical manifestations of septicemia and inoculated into blood culture bottles and incubated at 37 ° C in aerobic condition. Blood culture isolates were considered significant if results of blood culture bottles were positive within 24 to 48 h. The isolates were identified using standard procedures. The antimicrobial susceptibility testing was done by Kirby-Bauer disk diffusion technique as recommended by Clinical Laboratory Standards Institute (CLSI).

Results: Of 491 samples, there were 73 (14.8 %) positive blood culture.Infections were found to be more common in male Infants (54.7%) than in female infants(45.2%).The incidence of gram positive and gram negative organisms were 69.8% and 30.1% respectively.CoNS was the commonest isolate n=49 (67.2%), followed by Escherichia coli,Enterobacter and Acintobacterspecies each with 5 (6.8%) isolates. Pseudomonas, Klebsiella and Streptococcusspecies were other recovered isolates. Most CoNS were resistant to cephalexin (95.9%), erythromycin (93.8%), and cloxacillin (85.7%), and remained susceptible to vancomycin(100%), chloramphenicol(75.5%) and gentamycin (32.6%). There was high sensitivity to ciprofloxacine (54.5%), amikacin (31.8%) and gentamycin (27.2%) and relatively high resistance to cephalexin (100%), cefixime and tetracycline (95.4%) among gram negative isolates.

Conclusion: Study of bacteriological profile and their antibiotic sensitivity pattern plays a significant role for effective management of neonatal septicemia. As a result appropriate antibiotic prescription would minimize the risk of severe morbidity and mortality. Our data show that coagulase-negative staphylococcus can be an important cause of septicemia in newborn infants.

Keywords: septicemia , coagulase negative staphylococcus , neonatal , blood culture

P924: **Application of VNTR17 and VNTR23 in genotyping of Iranian Bacillus anthracis isolates, an improving amendment to the standard MLVA 8 typing system**

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Background and Aim: Iran is known to have experienced the largest ever-seen sheep epidemic of anthrax in the world as it lost l million animals in 1945. Despite a massive national vaccination scheme of farm animals, anthrax still occurs in some parts of Iran and causes outbreaks. Bacillus anthracis is an extremely clonal pathogen with a highly homogeneous population. Only a limited level of diversity has been detected in the genome of this bacterium. Tandem repeats (TRs) are among the few genetic markers that are understood to enable detection of genomic diversity between B. anthracis clones and sub-clones. Over the last decade, an increasing number of TRs have been used in Multiple Locus Variable number tandem Analysis (MLVA) systems to genotype B. anthracis. In the work presented here, VNTR17 and VNTR23 have added to the standard MLVA 8 strain typing method to assess their capability for strain typing of Iranian B. anthracis isolates.

Methods: Boilates were prepared from fresh cultures of 14 B. anthracis isolates. Recently published primers were employed for PCRs. Two individual PCR reactions sharing a single amplification protocol, were used to amplify VNTR17 and VNTR23 genomic stretches of 12 filed Iranian isolates, one Vaccinal strain (Sterne 34F2) and also one laboratory strain (Pasteure 17JB) of B. anthracis. To support the gel electrophoresis results, all the PCR amplicons were also sequenced.

Results: All the studied isolates/strains amplified PCR products. At VNTR17, two alleles of 386 bp and 452 bp were detected. Similarly, with VNTR23 a 514 bp-long product along with a larger allele of 577 bp were observed. In total, 3 combinational patterns of the two loci were identified in the study panel including 386/514, 386/577 and 452/577. This simple double-locus typing method enabled differentiation of Sterne 34F2 strain as 386/577 was exclusively represented by this strain.

Conclusion: In the years to come, we will observe more epidemiological studies using MLVA method on B. anthracis from Iran . While using standardized MLVA loci is necessary to harmonize such studies, we assume VNTR17 and VNTR23 provide a satisfactory level of discrimination that makes them good candidates for MLVA typing of Iranian B. anthracis population.

Keywords: B.anthracis, MLVA,VNTR17,VNTR23,Genotyping

P925: **Evaluation of fungal flora of Khorramabad hospitals with emphasize on AFs and CPA mycotoxins producer Aspergillus section flavi**

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Background and Aim: Background: Due to the substantial technological progress,the incidence of fungal infections has increased dramatically.Therefore hospital environment requires special attention to monitor the fungi.Air fungal load and predominant species in hospital environment influences the nosocomial fungal infections incidence indirectly.Mycotoxins are produced by many species of Aspergillus especially section flavi ,which leads to cause mycotoxicosis. The ultimate goals of this study were to evaluate the fungal flora and investigate toxigenic Aspergillus Section Flavi in hospital environments.

Methods: Materials and Methods: In this study air,surfaces samples using settle plate and swabbing methods respectively and soil samples from 5 hospitals were cultured on PDA , MEA and AFPA medium.Fungi were identified based on macro and microscopic features.For the Aspergillus section flavi qualitative analysis of cylopiazonic acid(CPA) and aflatoxins (AFs)were performed using thin-layer chromatography method.

Results: Results: Total of 707 fungal colonies including Penicillium,Cladosporium,Aspergillus,Fusarium,Alternaria,Rhodotorula spp and Cryptococcus neoformans were recovered.The most frequent Aspergillus species were A.niger followed by A.ochraceus A.flavus ,A.tammarii,and A.terreus.Sixty three out of 146 isolated colonies of Aspergillus were identified as A.flavus.These isolates were classified into four chemotypes based on their ability to produce AFB1 and CPA.

Conclusion: Conclusion: In terms of public and occupational health mycotoxicosis and nosocomial fungal infections ,isolation of unusual fungi particularly AFB1 and CPA producer toxigenic Aspergilli indicates encounter with new threats .Hence , a new approach of preventative measures ,monitoring and control of contact with airborne fungal conidia is necessary.

Keywords: Key words: Khorramabad hospitals, Aspergillus flavus, Aflatoxins, Cyclopiazonic acid

P926: **Molecular characterization of class I integron in Gram-negative bacteria isolated from patients with cystic fibrosis at Tehran hospitals by PCR**

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Background and Aim: Cystic fibrosis is a genetic disease. It Causes defects in the gene encoding a membrane protein is which regulatory. Because of not having enough efficiency in this regulatory proteins, sticky mucus is accumulateds between cells and pulomonery sillia .The aim of this study is assessing of molecular characterization of class I integron in Gram-negative bacteria isolated from patients with cystic fibrosis at Tehran hospitals by PCR

Methods: 52 suction samples of cystic fibrosis patients hospitalsed in Tehran hospitals were taken from sptember 2011 till June 2012. Fifty-five of Gram-negative bacteria identified with biochemical test . Family of beta-lactam and Aminoglycosides antibiotics using the disk diffusion test and mic method of antibiotic-resistant bacteria were identified by screening. The next step to determine the integron classes I , DNA extracted with the use specific primers of screening strains of integron class I and integron variable regions were shown by PCR

Results: The study on patients with respiratory infections in cystic fibrosis patients in Tehran hospitals in age from one and a half months to sixteen years, the results showed that the 55 isolates were detected Percentaye. Pseudomonas aeruginosa (%44), , Enterobacteriaceae SPP(%11), Achromobacter SPP(%14.5) ,Klebsiella SPP(%11), E.coli(%7), Stenotrophomonas maltophilia (%7), Acinetobacter SPP(%5.5), Aeromonas SPP(%1). These 55 strains, 18 strains have been screened for class I integron were. Then different patterns of class I integron and integron variable regions were amplified using the specific primers.

Conclusion: Withassessing and studing on hospitalised cystic fibrosis patients in Tehran hospitals with ages ranging from 1.5 to 16 years ,prevalence of bacterias different with considering their ages . Pseudomonas aeruginosa in the most frequent isolated bacteria in this patients. Subtyping the isolates carrying class 1 integron. There was correlation between the presence of integron and resistance to aminoglycosides and cephalosporins

Keywords: cystic fibrosis, bacteria, microbial prevalence,integron

P927: **Detection of Extended Spectrum β-Lactamase, AmpC β-Lactamase and Carbapenemase Produced by E.Coli and Klebsiella spp. in clinical isolates/Babol/Iran**

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Background and Aim: Beta-Lactame antibiotics selectively inhibit cell-wall synthesize, and widely used against gram positive and negative bacteria. Enterobacteriacea are one of the most significant reasons of community and acquired, as well as nosocomial infections. Resistance to β-Lactams could be mediated by producing Extended Spectrum β-Lactamase , AmpC β-Lactamase and carbapenamase in these bacteria. Prevalence of β-Lactamases producing E.coli and klebsiella spp. is widespread and genotyping method is not routine in clinical laboratories; therefore, this study aimed to detect Extended Spectrum β-Lactamase, AmpC β-Lactamase and Carbapenemase Produced by E.Coli and Klebsiella spp.

Methods: This cross-sectional study performed during 6 months. 259 isolated E.coli and Klebsiella spp. from clinical specimens were transferred to paramedical faculty microbiology lab. Antimicrobial susceptibility test (Disk Diffusion method) was applied following determination of these bacteria by API 20E strip test. To detect ESBL ceftazidime and cefotaxime disks (alone and with clavolanic acid); AmpC β-Lactamase ceftazidime disks (alone and with 3-amino phenylboronic acid in 300µg concentration); Carbapenemase ceftazidime disk and a disk contains of 2µl, 2-Mercaptopropionic acid were used. For all procedures, Mueller-Hinton agar medium in 35oc incubation temperature applied. Data was analyzed by SPSS 18

Results: Findings showed that from 259 isolated E.coli and 35 Klebsiella (Klebsiella pneumonia and oxytoca), 150(51%) for ESBL, 20(6.8%) for AmpC β-Lactamase and 84(28.6%) for carbapenemase were positive. More than one third (84) of E.coli produced 2 types of β-lactamase, and ESBL-carbapenemase producers had the highest percent of them, 68(81%). Results clarified that AmpC β-Lactamase has not been produced by Klebsiella spp. Furthermore, just 4(13.3%) Klebsiella pneumonia produced carbapenemase which just one of them co-existed with ESBL production.

Conclusion: With regards to high percent of Klebsiella pneumonia and E.coli producer ESBL and carbapenemase, it seems necessary to perform similar studies in other clinical and research centers of Iran. Moreover, genotypic method is recommended to determine β-Lactamases in this region.

Keywords: E.coli, ESBL, AmpC beta-Lactamase, Carbapenemase , Klebsiella

P928: **Tuberculosis: New Methods for Diagnosis**

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Background and Aim: Tuberculosis (TB) remains a major public health concern in most low-income countries. Hence, rapid and sensitive TB diagnostics play an important role in detecting and preventing the disease. In addition to established diagnostic methods, several new approaches have been reported.

Methods: A) The early method: 1) subcutaneous injections of tuberculin. 2)Chromatographic methods. 3)solid mediacultures such as Lowenstein—Jensen and Ogawa.B) the new methods: 1) The aim of the present work is to develop an immunoassay for the diagnosis of TB infection, using synthetic peptides of antigen (Ag) 85 complex of Mycobacterium tuberculosis (Mtb) H37Rv.2) In other test they evaluated a simple, robust and cost-efficient in-house DNA extraction and downstream Taqman approach for detection and quantitation of Mtb genomes from sputum of newly diagnosed TB patients and non-TB controls. 3) One prominent and readily available approach is to detect proteins that Mycobacterium tuberculosis secretes, such as Mpt64, the 6-kDa early secreted antigenic target (Esat6), the 10-kDa culture filtrate protein (Cfp10), and the antigen 85(Ag85) complex..4) A modified acid-fast staining method was developed for rapid detection of Mycobacterium tuberculosis and its L forms, where in carbolfuchsin and dioxogen were mixed into the sputum smear. With this method, the dyeing time is shortened and heating is not required. The sensitivity, specificity, positive predictive value, negative predictive value, positive rate, and diagnostic efficiency of the new method were compared to those obtained by PCR using 50 clinical samples.

Results: The cost factor need not restrain laboratories from adopting molecular methods for diagnosis of tuberculosis since these methods have advantages of speed, sensitivity, and specificity.

Conclusion: Conclusions and future perspectives: Timely diagnosis of TB is essential because prompt and specific treatment, as early as possible, is important in ensuring a favorable clinical outcome.

Keywords: Tuberculosis ,antigen 85 complex ,immunoassay,DNA

P929: **Antibiotic Resistance Profiles, Methicillin Resistance, and Presence of mecA Gene in Clinical isolates of Staphylococcus aureus.**

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Background and Aim: Staphylococcus aureus is a major cause of both hospital and community acquired infections. Increasing of methicillin-resistant Staphylococcus aureus (MRSA) in recent years, has led to severity of disease caused. The high cost of antibiotics and increasing resistance to them, justify the identification, prevention and control of these infections. The purpose of this study was to evaluate methicillin-resistant Staphylococcus aureus isolates with three methods, namely oxacillin disk diffusion, oxacillin agar screening and PCR.

Methods: A total of 183 isolates of Staphylococcus aureus were collected from clinical samples of four selected hospitals in Tehran, Tabriz and Shabestar, Iran (Children's Hospital of Tehran, Imam Reza and Aalinasab Hospital of Tabriz and Fatemiyyeh Hospital of Shabestar). The isolates were identified by using the conventional biochemical tests (catalase, coagulase, mannitol fermentation and the deoxyribonuclease production). Three methods of oxacillin disk diffusion, oxacillin agar screening and PCR (for mecA gene) were applied to determine susceptibility to oxacillin. Conventional disc agar diffusion test was used to evaluate the antibiotic sensitivity of our isolates against 10 antibiotics according to Clinical and Laboratory Standards Institute (CLSI).

Results: The number of Staphylococcus aureus isolates which were MRSA by oxacillin disk diffusion method recorded as 86 (47%). Oxacillin agar screening test containing 0.6µg/ml oxacillin could detect 107 isolates (58/5%). The same test containing 4µg/ml oxacillin could detect 77 isolates (42/1%) and by using 6µg/ml oxacillin 75 isolates (41%) were detected. The mecA gene (533 bp) was detected in 77 isolates (42/1%) by PCR. Antibiotic resitance obtained for other antibiotics were as follows: penicillin (97.8%), rifampin (20.8%), erythromycin (50.3%), clindamycin (36.1%), cefazolin (32.2%), ceftriaxone (48.1%), cotrimoxazole (54.1%), gentamicin (38.3%) and ciprofloxacin (36.6%). All of the test isolates were susceptible to vancomycin.

Conclusion: Our results revealed that the agar screening test containing 4 µg/ml of oxacillin and incubation period of 24 h was superior to other phenotype-based susceptibility tests used in this study, with sensitivity and a specificity approaching 100%. Thus in the absence of molecular techniques, this was the best predictor of methicillin resistance in S. aureus among the tests perfomed in this study.

Keywords: Staphylococcus aureus, MRSA, oxacillin agar screening, PCR, mecA

P930: **Pattern of antibiotic resistance in Klebsiella pneumoniae isolated from clinical specimens obtained from patients attending hospitals in Shahrekord**

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Background and Aim: Klebsiella pneumoniae is a Gram-negative Enterobacteriaceae family causing septicemia, pneumonia and urinary tract infection .Klebsiella bacteremia and pneumonia caused most deaths due to abuse of the disease increases and the main reason for the emergence of antibiotic-resistant strains of antibiotics.

Methods: In this study, 173 isolates of Klebsiella pneumoniae were isolated from patients admitted to hospitals in Shahrekord city . Sampels were identified with routine tests. Then, by the method of antibiotic susceptibility test Disk diffusion was performed according to CLSI criteria.

Results: Among the 173 strains of Klebsiella pneumoniae isolated, 56.6, 54.3, 71.1,79.2 precent of isolates was resistance to Amoxicilin, Nitrofurantoin, Cotrimoxazole and Cefazolin. In other hands , 65.9, 75.7,76.9,78 precent of isolates was susceptible to Ciprofloxacilin , Imipenem, Amikacin and Gentamicin.

Conclusion: Acordint to high rates of resistance isolets in this study , we advice that antibiogram test do exactly for select aproprate antibiotic to prevent costly to the health system

Keywords: Klebsiella pneumonia, Disk diffusion , bacteremia

P931: **High prevalence of class 1 and 2 integron and its concomitance with Quinolone resistance and ESBL production among Escherichia coli and Klebsiella pneumoniae: A prospective study**

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Background and Aim: Antibiotic resistance among bacteria such as Escherichia coli and Klebsiella pneumoniae isolated from the patients after surgical operations or community acquired infections causes high concern. High prevalence of quinolone resistance in these bacteria has been described in Europe, South America and Asia, and Middle East. The Middle East regions showed a significant increase in antibiotic resistant, but until few years back maintained low rates of resistance. In K. pneumonia and E. coli, quinolone resistance is frequently found among strains producing extended-spectrum β-lactamase (ESBL) than among ESBL-negative strains, which may be related to the coordinated expression of several mechanisms of resistance. Though ESBL problem was first reported from Europe in 1983. Since that, it involved all over the world and posed a major clinical dilemma. In recent years, it has been shown that a substantial portion of the resistance genes present on plasmids and transposons in gram-negative bacilli are integrated in DNA elements called integrons, which are responsible for their dissemination. Most of the resistance integrons found to date in clinical isolates of Enterobacteriaceae are class 1 integrons, which are highly associated with resistance to antimicrobial agents.An upward trend in the incidence of quinolone resistance was observed in last four years in E.coli and K. pneumoniae clinical isolates in Sina hospital, a 150 bed teaching and referral hospital for Northwest Iran. Thus, the objective of the present study was to investigate whether integrons contribute to this resistance and even emergence of ESBL and / or multidrug resistance in these isolates recovered from various clinical infections from high risk group of patients. The integron was chosen as a marker of transfer because it is a well-defined transferable genetic determinant.

Methods: During a period of nine months, 170 E.coli and K.pneumoniae were isolated from various clinical infections and 120 of them were (63 E.coli and 57 K. pneumoniae) found resistance to ciprofloxacin and ofloxacin and or nalidixic acid on disk agar diffusion test. These isolates were tested for susceptibility to the other antimicrobial drugs and Extended spectrum beta lactamase production as described by the Clinical and Laboratory Standards Institute and later stored at –70°C . Isolates were analyzed by Multiplex PCR technique to determine various classes of integrons, following which the products were analyzed by gel electrophoresis.

Results: Among fluoroquinolone (class 1 and 2) resistant isolates , a positive test result for class 1 integrons was found for 34.4% E. coli and 54.8% K. pneumoniae isolates, while presence of Class 2 integron was observed in 65.5% of E.coli and 29% of K. pneumoniae. 70% of class 1 integron possessing E.coli and K. pneumoniae were ESBL producers. A positive test result in these isolates was significantly associated with non-susceptibility to other antibacterial drugs, with significant resistance toward ?-lactams, including ceftazidime ,cefotaxime, cefepime ,cefpodoxime.

Conclusion: The findings suggest that the mechanism of resistance to fluroquinolones and β-lactams is related to integron carriage and is a real concern.

Keywords: Escherichia coli, Klebsiella pneumoniae, Quinolone-resistance,ESBL, Integrons

P932: **Identification of Acinetobacter baumannii isolates, producing TEM and SHV Expanded Spectrum β-lactamase (ESBL) at Tehran hospitals by molecular method.**

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Background and Aim: Many studies show that multidrug-resistant Acinetobacter baumannii is responsible for producing several problems in different parts of the hospital specially ICU, and owns extended-spectrum beta-lactamases (ESBL). ESBLs are encoded by different genes, including blaTEM and blaSHV. Concerning the importance of infection reports caused by these bacteria in our country during recent years, the aim of this study is to identify the genes encoding the beta-lactamase TEM and SHV by using PCR method among 100 isolates of A. baumannii taken from patients in hospitals of Tehran.

Methods: In this study, a total number of 100 clinical isolates A. baumannii were used. Isolates were diagnosed at first, using culture methods and biochemical tests, and then the antibiogram was performed towards 11 different antibiotics by disk diffusion method based on CLSI guidelines. Phenotypic identification for ESBL production was confirmed by double disk synergy test and PCR for determining the prevalence of blaTEM and blaSHV was eventually performed using specific primers for all the isolates.

Results: Results of antimicrobial susceptibility testing showed that most of the antimicrobial resistance is respectively related to Amikacin, Cefepime, Piperacillin-tazobactam, Imipenem, Meropenem, Gentamicin, Tobramycin, Tetracycline, Ampicillin-Sulbactam and the less resistance belongs to Polymyxin B. According to the double disk outcome synergy test, 20% of isolates were also producing broad-spectrum lactamases. The prevalence of TEM and SHV were respectively as follow: TEM: %56 and SHV: %63

Conclusion: The above research shows an increase in ESBL production; moreover the prevalence of blaSHV gene among the A. baumannii isolates is more than the blaTEM.

Keywords: A. baumannii, ESBL, blaTEM, blaSHV

P933: **Effect of the Disinfectant and Antiseptic Agents on Standard and Clinical Resistant Strains (Acinetobacter and Pseudomonas Species) Isolated from Patients**

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Background and Aim: Disinfection is removed of microorganisms in the vegetative form, irrespective of their pathogenic character, from inanimate goods and surfaces; with possible removal of spore bacteria. Due to the growing number of outbreaks of infection causing multi drUg resistant (MDR) bacteria in hospital, it becomes essential to set up a sanitation program that indicates that the appropriate chemical agent was chosen for application in the most effective way. Validating the effectiveness of decontamination and disinfection is an important and often challenging task. The aim of this study was to evaluate the efficacy of common disinfectant solutions (Formaldehyd, Glutardialdehyde, povidon iodine, Hydrogen peroxide) and Microzed (HD, GP-H, ID-MAX) against standard strain and clinical resistant strains obtained of hospitalized Patient.

Methods: In first step, standard strains (E.coli, S. aureus, P. aeroginosa, B. subtilis) purchased of IROST and clinical strains Acinetobacter, Pseudomonas (MDR), obtained of hospitalized patient were isolated and identified. In following were determined the minimal inhibitory concentration (MIC) and Minimum Bactericidal Concentration (MBC) of each disinfectant solution against the studied strains.

Results: With study and compare the behavior of clinical strains (MDR) with standard strains, we find that MIC and MBC value of each disinfectant against clinical strains were the same to the MIC and MBC value for standard strains.

Conclusion: In conclusion, According to the results, the aforesaid disinfectants can be used to cope with the Clinical Resistant Strains also like those dilutions of the standard species, specially Microzed types (HD, GP-H, ID-MAX),so It can be widely used.

Keywords: Disinfectant, Clinical strain, Acinetobacter, MDR, MIC, MBC,hospital infection

P934: **Antimicrobial Synergic Effect of Allicine (2-Propene-1-sulfinothioic acid S-2-propenyl ester) and Silver Nanoparticles on Skin Infection caused by Methicillin-Resistant Staphylococcus aureus strains**

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Background and Aim: Today, the commonly used antibiotics may be ineffective to induce resistance against multiple pathogens. As a result an effort to find a new approach for solving the issue has been considered. The aim of this study is to investigate antimicrobial properties of allicin (2-Propene-1-sulfinothioic acid S-2-propenyl ester), silver nanoparticles and their combinations against skin infection caused by Methicillin-Resistant Staphylococcus aureus (MRSA) strains in animal models.

Methods: In this study, the effects of allicin, silver nanoparticles and their combinations were investigated on 18 mice in which the skin infection was caused by MRSA bacteria. In vitro the Minimum Inhibitory Concentration (MIC) of bacterial growth and Minimum Bactericidal Concentration (MBC) of allicin, silver nanoparticles, and their combinations were determined by microdilution technique. In vivo (animal models) Staphylococcus aureus number were counted based on CFU/ml fourth day after treatment.

Results: The results of in vitro assays showed that MIC of allicin and silver nanoparticles on MRSA were 2.2 mg/ml, 5.6 mg/ml, respectively and also MBC of allicin and silver nanoparticles on MRSA were 3.1 ppm and 7.5 ppm, respectively. However, MIC and MBC of allicin and silver nanoparticles together on MRSA were 0.4 mg/ml and 1.1 ppm, respectively. The result of in vivo tests on skin infection showed that number of bacteria counted for control, silver nanoparticles, allicin and their combinations were 377×108, 80×106, 43×105 and 0, respectively.

Conclusion: The results clearly indicate that allicin and silver nanoparticles when used in combination exhibit a synergistic effect. Thus, the present results obtained can be contemplated in future to expedite and improve the treatment of skin infections.

Keywords: Antimicrobial synergic, allicin, silver nanoparticles, skin infection, Methicillin-Resistant Staphylococcus aureus (MRSA) strains.

P935: **In vitro antifungal susceptibility of Candida glabrata isolated from oral cavity of HIV+ individuals to Fluconazole and Voriconazole**

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Background and Aim: Following the widespread use of immunosuppressive therapy and broad-spectrum antimycotic prophylaxis, C. glabrata has emerged as an important opportunistic pathogen in the oral mucosa. In addition, C. glabrata possesses both innate and acquired resistance against antifungal drugs, due to its ability to modify ergosterol biosynthesis, mitochondrial function, or antifungal efflux. This resistance allows for its relative overgrowth over other susceptible species and may contribute to the recent emergence of C. glabrata infections in immunocompromised populations. In this study the antifungal susceptibilities of 20 oral Candida glabrata isolates to fluconazole and voriconazole were compared.

Methods: Twenty C. glabrata isolates from Oral cavity of HIV+ patients were evaluated. These strains were previously identified by germ tube testing, plated on CHROMagar Candida medium , and molecular methods. In Vitro antifungal susceptibility was determined using the microdilution method described in the CLSI M27-A2 guideline and MICs of both fluconazole and voriconazole were determined at 48 h for each isolate.

Results: The MIC90s of voriconazole and fluconazole were determined to be 1 and 32 µg/ml, respectively. The voriconazole MICs were >1 µg/ml for 3.1% of the isolates tested. The average voriconazole MIC for isolates susceptible to fluconazole (MIC, <8 µg/ml) was 0.257 µg/ml, the average voriconazole MIC for isolates that showed dose-dependent susceptibility to fluconazole (MIC, 16 to 32 µg/ml) was 0.965 µg/ml, and the average voriconazole MIC for isolates resistant to fluconazole (MIC, >32 µg/ml) was 3.333 µg/ml. Data from our study also show that C. glabrata isolates for which the fluconazole MICs were significantly elevated were also those for which the voriconazole MICs were elevated.

Conclusion: These data may imply that voriconazole would not be effective in the treatment of infections caused by C. glabrata isolates for which the fluconazole MICs are elevated, Therefore, clinical trials need to be performed to evaluatethe efficacy of voriconazole against C. glabrata oral infections.

Keywords: Candida glabrata, Fluconazole and Voriconazole

P936: **Prevalence of parasitic agents causing diarrhea in children under ten years of Clinical laboratories isolated city of Ilam**

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Background and Aim: Agents causing diarrhea in children include viruses, bacteria, parasites and fungi. Giardia, Cryptosporidium, Entamba histolitica, Blastosistis hominis, and the most common causes are parasites. This study the prevalence of parasitic diseases in children under 10 years and its causes in the city of Ilam evaluated.

Methods: In this study, in the 6-month stool samples of children under 10 years referring to clinical laboratories were obtained in Ilam city. To identify parasites by direct smear method, formalin-ether and modified acid fast staining was performed .The criteria for determining the parasite infection, was at least presence 1 parasite in the sample.

Results: The results of this study showed that in total 530 samples , Entamoeba histolytica 24 (4.5%) , Giardia lamblia12 (2.3%) and from non-pathogenic protozoans ,Entamoeba coli (4.23%) percent, Endolimax nana(3.4%) percent and Iodomoeba butschli (5.5 %)percent reported

Conclusion: In this study it observed that Entamoeba histolytica is prevalent among the most pathogenic protozoa and Entamoeba coli is prevalent as the non-pathogenic protozoan and it can be regarded as an indicator of regional health. More such studies may be necessary to increase the surface area of health.

Keywords: 1-Diarrhea 2-parasite 3- Entamoeba histolytica

P937: **Prevalence and Molecular Characterization of AmpC-producing Clinical Isolates of Escherichia coli From Zahedan province, Iran**

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Background and Aim: AmpC enzymes are inducible and can be expressed at high levels by mutation. This study was undertaken to characterize the AmpC producing clinical isolates of Escherichia coli.

Methods: E. coli isolates recovered from three major hospitals in zahedan, southeastern Iran, were selected on the basis of a resistance phenotype to third-generation cephalosporins and cefoxitin. Phenyl boronic acid as inhibitor with cefoxitin was used to confirm expression of AmpC. The presence of genes encoding ACC, FOX, MOX, DHA, CIT and EBC were detected by multiplex PCR. The existence of mutations in the regulatory region of the chromosomal ampC gene were studied by PCR and sequencing.

Results: 13 of 392 E.coli isolates were selected as AmpC producer. 11/13 isolates contained a blaCMY-2 gene; 12/13 AmpC positive strains harbored changes in the promoter/attenuator region, which could explain increased expression of the chromosomal AmpC enzyme; In 10/13 strains both chromosomal and plasmid-mediated mechanisms responsible for AmpC production were found.

Conclusion: AmpC producing E.coli isolates cause significant resistance to cephalosporin. This work showed that hyper producing chromosomal ampC could be as frequent as plasmid-mediated mechanisms.

Keywords: AmpC β-lactamases - Amp C genotypes - cefoxitin resistance – CMY-2 - promoter /attenuator mutations

P938: **Surveying the leaf and stem extract of Pistacia Atlantica in treating process of wound infected by staff and wound caused by second degree burn in rats.**

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Background and Aim: Abstract: Aim background: the skin infected wound and wounds caused by burn are very epidemic in society , and these kind wounds rate treatment in effective to alleviate the patint , s sorrow . applying the herbal drugs with less complications to treat the wounds has attracted many people and researchers , attention , on the other hand , the evidences show that utilizing the leaf and stem of pistacia atlantica to treat the skin wound an akin burn traditionally is very common among indigenous people of Ilam and lorestan. Considering this significance , surveying the effect of leaf and stem of pistacia atlantica to treat skin wound and skin burn was devised and implemented .

Methods: Methods and materials: 42 head of rate of the same aye , vistar species , and 250 + 20 grams weight were used in this investigation , in 18 head of these rats , the wound infected by staphylococcus aureus and in 18 head , the second degree burn and wound infected by pseudomonas aeruginosa was constructed . every group of rats was classified into three clusters with six members . per group was treated by 5% eucerin(testifier group) , eucerin and water extract , eucerin and alcoholic extract obtained from leaf and stem of pistacia atlantica , the treatment was done two times in night and day (7 in the morning and 7 at night) during 24 days.

Results: Results: The result show that in vivo , treatment with the effect of water extract on the infected wound by staphylococcus aureus was better that the alcoholic extract (12 days for water extract and 15 days for alcoholic extract) , treating the second degree burn and wound infected pseudomonas aeruginosa (17 days) applying water extract was done faster than alcoholic extract (20 days).

Conclusion: the study cleared that the local using of water extract of pistacia atlantica causes to accelerate the treatment of wound and burn in laboratory rats in a very short time.

Keywords: Keywords: Extract leaf and stem , pistacia Atlantica wound , second degree burn , rats

P939: **Microbiological evaluation of dental unit water at dental offices and dental hospital in the city of khorramabad.**

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Background and Aim: Aim background: The quality of dental unit water is of considerable importance since patients and dental staff are regularly exposed to water and aerosols generated from the dental unit. Following ADA instruction, this study was performed to control the contamination of Dental Unit Water Line (DUWL) to less than 200 CFU/ml. The purpose of this study was to evaluate the microbiology of DUWL at dental offices and dental hospital of khorramabad.

Methods: Materials and Methods: In this laboratory study, DUWL of dental offices from different areas as well as that of the dental hospital of khorramabad was microbiologically evaluated. An amount of five ml water from the syringes and high speed hand pieces before and 2 minutes after flushing and drinking water of units and tap water were gathered in three different sterile polyethylene dishes. Then the samples were cultured on the specific media and the number of the bacterial colonies were counted after keeping at 37°c for 48 hours. The data were analysed by SPSS software and chi-square, Fisher's exact and Paired t-tests as well as ANOVA were used.

Results: Results: 33.3% of all species samples were positive for presence of bacteria. Microorganisms isolated were as follow: Staphilococcus aureus, coliform, E.coli, pseudomonas aeroginosa, klebsiella. The number of the colonies were more than the standard limit. Contamination of the water of hand pieces was reduced after flushing. Contamination of tap water compared to the water of handpieces was less and mean contamination of the samples gathered from the dental offices was higher than those gathered from the dental hospital.

Conclusion: Conclusion: Flushing decreases the contamination of DUWL, but in surgeries and in persons with immunedeficiency, the use of other methods of DUWL control and decontamination is recommended.

Keywords: Key words: Microbiology, biofilm, DUWL (dental unit water line).

P940: **Rapid and specific detection of verotoxin genes in Escherichia coli by the polymerase chain reaction.**

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Background and Aim: ABSTRACT Aim background: A set of four synthetic oligonucleotide probes derived from sequences of the VT1 (Shiga-like toxin I [SLT-I]) and VT2 (SLT-II) genes were used in a polymerase chain reaction (PCR) amplification procedure to detect these genes in some enteric pathogens.

Methods: Methods and materials: A total of 31 verotoxin-producing Escherichia coli strains and 35 isolates of other recognized enteric pathogens were studied. PCR amplification products identifying the VT1 and VT2 gene sequences were observed only in nucleic acid extracted from strains found to be VT positive in traditional tissue culture assays.Template nucleic acid extracted from other gram-negative bacteria was found to be negative with the exception of five isolates of Shigella dysenteriae type 1 in which good amplification with the VT1 probe was observed.

Results: Results: The oligonucleotide probes clearly distinguished VT1 and VT2 strains of E. coli and did not give specific amplification with nucleic acid from VTe (a SLT-II variant)-producing E. coli. VT1 or VT2 genes or both were not detected in E. coli K-12 strain C600 or HB101 or in strains known to express other virulence factors, such as enterotoxins, adhesins, hemolysins, or unrelated cytotoxins. The sensitivity of the PCR procedure for detection of both VT1 and VT2 genes was determined to be 1 ng of total nucleic acid.

Conclusion: Discussion: Furthermore, the VT1 gene was easily detected when only 100 pg of nucleic acid was used as the template in the PCR procedure.

Keywords: Key words: Escherichia coli , polymerase chain reaction , verotoxin

P941: **Antibacterial effects of silver nanoparticles and its combination with Zataria multiflora essential oil on Gram positive and Gram negative bacteria**

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Background and Aim: By the help of the antimicrobial resistant pathogens distribution, the scientists have attempted to substitute antimicrobial medicine with various nanoparticles and plant originated antibacterial substances. The aim of this study is to access the antibacterial effects of silver nanoparticles solely and in combination with Zataria multiflora essential oil on Staphylococcus aureus (MRSA, MSSA), Staphylococcus epidermidis, Streptococcus pyogenes and Pseudomonas aeruginosa.

Methods: In this study different concentrations of silver nanoparticles and Z. multiflora essential oil solely and in combination with each other were applied on bacterial growth culture by well diffusion method. For combination effects, three concentrations (minimum, medium and maximum) of silver nanoparticles and plant essential oil were determined and nine different combinations were made. Data were analyzed by Two-way analysis of variance. Level of significance was considered at $p<0.05$.

Results: The results of the experiment showed that silver nanoparticles and Z. multiflora essential oil solely have significant antimicrobial activity against bacteria in all concentrations. The inhibition zone in bacteria were increased when silver nanoparticles in combinations with the minimum concentration of the plant essential oil, but the inhibition zone were decreased when silver nanoparticles in combinations with plant essential oil in the range of medium and maximum of concentrations.

Conclusion: It seems the component of plant essential oil such as groups/molecules phenolic and alkaloids are reduced antimicrobial effects of silver nanoparticles.

Keywords: Silver nanoparticles, Zataria multiflora, antibacterial effects, Gram-positive, Gram-negative.

P942: **Culture of green cyanobacterium Spirulina sp. with Antibacterial activity**

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Background and Aim: Marine organisms including cyanobacteria and micro-algae, are novel sources of potentially useful bio-active compounds that represent in development of new pharmaceutical agents. Antimicrobial effect of green-Blue Cyanobacterium Spirulina sp. Extracts, were tested against some bacterial Test strains.

Methods: Different 5 media were applied for cultivation of this cyanobacterium. Crude extracts were subjected to screening by 2 methods against Gram-positive (Bacillus subtilis, Micrococcus loteus and Staphylococcus aureus) and Gram-negative (Escherichia coli, Enterobacter species, Klebsiella species, Proteus species, Pseudomonas species) test bacteria. Bio-active materials were collected by different methods from each of 2 microalgae species. Antibacterial activity was assayed using well diffusion and paper disc diffusion assay.

Results: This study shows that this species of Spirulina contain microbial growth inhibitors, and so, each Media and methods that were tried to extract compounds give us different results.

Conclusion: The results of this study indicate the potential use of marine cyanobacteria and micro-algal extracts, as a source of new antimicrobial compounds.

Keywords: Microalgae, Culture Medium, Extraction, AntiBacterial, Chlorella, Spirulina

P943: **Antimicrobial activity of Different parts of Lavandula Plant**

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Background and Aim: Lavandula (common name Lavender) is a genus of many species of flowering plants in the mint family, Lamiaceae, as evidenced by its bilabiate flowers, aromatic oils, opposite leaves and square stem. From 2500 years ago, Ancient Egyptians used Lavender in embalming, perfumes, cosmetics, and medicines and now it applies at many applications. In this study,we used this plant for studying of antimicrobial properties.

Methods: Extraction methods were performed from different parts of plant. Antimicrobial assay were applied for against 6 types test strains of bacteria consist of gram positive: Micrococcus, Staphylococcus, Bacillus, and gram negative: Escherichia coli, Klebsiella, pseudomonas: and 2 Yeasts: Saccharomyces and candida. The antimicrobial activity of different Extracts and essential oils was determined by using two methods, the agar well diffusion method and the disk diffusion method.

Results: Each extracts of this medicinal plants exhibited varying degrees of antimicrobial activity against an array of all Gram-positive bacteria. Experiments showed us no effect of different extracts against Gram-negative bacteria and yeasts.

Conclusion: Characterization and study of different extracts of this plant are promising a big potential for incorporation into various products for which a natural antimicrobial additive is desired.

Keywords: Lavandula, Antimicrobial activity, Extracts, Extraction, Essential oil

P944: **Molecular Detection and Speciation of Campylobacter spp . in Children Gastroenteritis using PCR-RFLP method in Karaj Hospitals**

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Background and Aim: Campylobacter spp. are recognized as one of the major causes of food borne diseases and infections. C. jejuni and C. coli are the most commonly reported species causing human bacterial gastroenteritis . In this survey PCR-RFLP technique was used for detection and simultaneous speciation of Campylobacter isolates in stool samples and determination of the frequency of Campylobacter contamination in the cases of children gastroenteritis in Bahonar hospital of Karaj city.

Methods: To do this two hundred stool samples were obtained from neonates and children up to the age of 8 years with gastrointestinal disorders during June to September of 2011. DNA of the samples were extracted using DNG plus kit (Cinnagen) and PCR was optimized to amplify a 491 bp fragment of the 23s rRNA gene of Campylobacter genus and C. jejuni RTCC 1097 reference strain as positive control in the clinical samples, then RFLP technique using AluI and TasI was performed to differentiate jejuni, coli, lari and upsaliensis species.

Results: Evaluation of PCR positive samples (21 out of 200/10.5% of the samples) showed the jejuni species electerophertic pattern in 11(5.5%) and coli species pattern in 7(3.5%) of the samples, three out of 21 positive samples (1.5% of total samples) showed both of the patterns and mixed infection.

Conclusion: According to the results of this survey it was concluded that PCR-RFLP technique can be used as a rapid , sensitive and specific method for detection and simultaneous differentiation of Campylobacter species in clinical samples.

Keywords: Campylobacter, children stool, PCR, PCR-RFLP

P945: **The Activity Study of Epstein-Barr Virus in Multiple Sclerosis**

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Background and Aim: The Epstein–Barr virus (EBV), also called human herpes virus 4 (HHV-4), is a virus of the herpes family, and is one of the most common viruses in humans.EBV can infect a number of different cell types, including B cells and epithelial cells. Recent studies indicate that EBV-specific cellular and humoral immune responses and the regulation of viral persistence in EBV-infected memory B cells are altered in patients with autoimmune diseases. EBV is the causative agent of acute infectious mononucleosis (1). Multiple sclerosis (MS) is believed to be an immune-mediated disorder.MS is a chronic inflammatory disease of the CNS that leads to demyelination and neurodegeneration. The causes of MS are not known, but several factors have been shown to be associated with the risk of the disease, including certain genes, vitamin D, smoking and Epstein Barr virus infection(2). The aim of this study was to evaluate the effect of the virus EBV on multiple sclerosis.

Methods: According to research done, review the amount of IgG level virus EBV ELISA method and molecular methods of serum samples of MS patients is suggested.

Results: EBV infection increases the risk of MS before onset of MS is high EBV antibody titers. MS onset disease may be associated with EBV infection, the average time between the onset of MS 4 years. Capsid antigen IgA against Virus Capsid Antigen (VCA) and IgG against VCA. Nuclear antigen EBNA complex, EBNA-1,2. T-cell responses to EBV infection could include clones that are potentially cross-reactive with self-antigens. Indeed, T-cell cross-recognition between EBV antigens and myelin basic peptides has been demonstrated (3).The strongest predictors of MS were serum levels of IgG antibodies to EBNA complex or EBNA-1. Suggesting that EBV may be a risk factor for autoimmune diseases (4). In summary, these findings demonstrate an increased immune response to EBV in MS patients, which suggests that the virus plays an important role in the pathogenesis of disease. Further studies are necessary to clarify whether targeting EBV or the immune response against the virus may become therapeutic options in MS .

Conclusion: These results suggest, antigens EBV (VCA, EBNA-1) are elevated in individuals with MS and relationship between EBV infection and development of MS. According to research done, the proposal based on the prevention of diseases caused by the invading viruses to the nervous system and controlling the predisposing factors of MS disease. 1. Brooks, G.; Butel, J.; Carroll, K.C; Morse, S.; Mietzner, T. (2010): Jawetz, Melnick, & Adelberg's Medical Microbiology, Twenty-Fifth Edition: McGraw-Hill Companies,Incorporated. Available online at http: //books.google.com/books. 2. Sospedra, Mireia; Martin, Roland (2005): Immunology of multiple sclerosis*. In Annu. Rev. Immunol. 23, pp. 683–747. 3. Pohl, D.; Krone, B.; Rostasy, K.; Kahler, E.; Brunner, E.; Lehnert, M. et al. (2006): High seroprevalence of Epstein–Barr virus in children with multiple sclerosis. In Neurology 67 (11), pp. 2063–2065. 4. Kleinschmidt-DeMasters, B. K.; Tyler, Kenneth L. (2005): Progressive multifocal leukoencephalopathy complicating treatment with natalizumab and interferon beta-1a for multiple sclerosis. In New England Journal of Medicine 353 (4), pp. 369–374.

Keywords: virus infection,multiple sclerosis, VCA, EBNA

P946: **Cloning and Molecular Characterization of an Immunogenic LipL41 Protein of Leptospira interrogans Vaccinal Serovar Sejroe hardjo**

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Background and Aim: Leptospirosis is an emerging infectious disease and is considered to be the most widespread zoonotic disease in the world. This disease caused by Leptospira spp. LipL41 is the immunodominant antigen recognized during the humoral immune response to leptospirosis.The cloned gene could be further used for expression of recombinant protein for serodiagnosis.The LipL41 protein could be a useful diagnostic tool for the detection of leptospirosis The aim of this study was Cloning and Molecular Characterization of an Immunogenic LipL41 Protein of Leptospira interrogans vaccinal Serovar Sejroe hardjo

Methods:: Leptospira interrogans serovar Sejroe hardjo (LSH-RTCC2821) was used in this study which obtained from the Leptospira Reference Laboratory, Razi Vaccine and Serum Research Institute, Karaj, Iran.The bacteria were inoculated into selective culture medium EMJH and the Genomic DNA was extracted by standard Phenol-Chlorophorm method.The lipL41 gene was amplified by specific primers. PCR products of pathogenic serovar Sejroe hardjo was ligated in pTZ57R/T vector and transformed in competent E.coli Top10 cells.The extracted recombinant plasmid was sequenced.Homological analysis was performed by BLAST and phylogenetic analysis against the lipL41 nucleotide sequence database on the GenBank was done with Neighbor-Joining (NJ) optimality criteria of MEGA4 software and MegAlign program.

Results: PCR amplification of the lipL41 gene resulted in an 1065bp lipL41 gene product. The sequence was deposited in the GenBank database.The percentage identity and divergence among different leptospiral serovars was deduced using the Blast programme.DNA sequence analysis revealed that vaccinal serovar Sejroe hardjo (LSH- RTCC2821) was most closely related to field serovar Sejroe hardjo(LSH-RTCC2810)with (99.5%identity).The serovar Sejroe hardjo (accession No: AY642286) was more distantly related (99.3%identity).

Conclusion: The results showed that the lipL41 gene was highly conserved among pathogenic Leptospira serovars (>90% idenditity). In conclusion, the protein expressed by lipL41 gene may be used in diagnostic methods like ELISA and also can be a good candidate for recombinant vaccine against leptospirosis.

Keywords: leptospirosis,lipL41 gene,cloning, Sejroe hardjo

P947: **Prevalence of Superficial and Cutaneous Mycoses among Student Residents in Semnan University of Medical Sciences Dormitory in 2011**

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Background and Aim: Superficial and cutaneous mycoses are especially prominent in communal areas such as university dormitories. The fungal mycoses are contagious and can be spread from person to person.In crowded communal area such as dormitories, fungal mycoses specially athlete's foot can spread easily. The main aim of this study was to determine the prevalence rate of both superficial and cutaneous mycoses among student residents in dormitories of Semnan University of Medical Sciences.

Methods: Formany superficial skins, a clinical examination of the affected person and microscopic examination of the sample may be sufficient to determine that a fungal infection is present. Fungal specimens were obtained and collected in the dormitory hall. The skin scrapings were gently removed from the skin edge surface using a blunt scalpel. Sticky tapes (transparent tapes) were used to take the specimens in superficial mycoses e.g. tinea versicor. To make a further analysis, the fungal specimens were brought to the mycology laboratory located at faculty of medicine. The KOH preparations were used to identify fungal organisms in the skins and hairs. Fungal cultures such as Sabouraud's dextrose agar with chloramphenicol and cycloheximide and etc. were used to identify the specific fungi present.

Results: Three dormitories was investigated, two for females and one for males. Totally, 704 student residents were visited for fungal infections. 127 (18%) were suspected of having superficial and cutaneous mycoses. The prevalence of cutaneous mycoses and superficial mycoses among the suspect students were 7.08% (9 cases) and 67.71% (86 cases) respectively.The majority of patient suffered from the dandruff or seborrhea of the scalp and then to the tinea pedis. Trichophyton rubrum(5 cases) and Epidermophyton floccosum (2 cases) were the causative agents of tinea pedis among the student residents.

Conclusion: It seems the fungal mycoses are still common in the communal area such as dormitories of universities in our country due to the high crowded student's resident. This study showed that long stay students in the dormitories were contaminated more than those had short stay. Athlete's foot was the prominent fungal mycoses among the student residents. Since athlete's foot spreads easily and it almost spreads by walking bare foot on contaminated surfaces, it is strongly suggested that the dormitories' floors be cleaned using an appropriate disinfectant regularly. Dandruff or pityriasis capitis was the prominent superficial mycoses among the student residents. As a result, a normal anti-dandruff shampoo that contains zinc pyrithione or coal tar may clear with soap and water helps to remove the greasy sebum from the hair.Theearly screening for fungal mycoses among the students especially those who new registered to dormitories are recommended. Finally, it is recommended to the all student residents to pay more attention to their individual hygiene in the dormitories.

Keywords: Superficial and Cutaneous Mycoses, Dormitory,Students Resident

P948: **Survey of efficacy and functional impact of disinfectant agent on computer keyboards in Fatemiyeh and Amiralmomenin hospitals of Semnan city**

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Background and Aim: One critical factor for the transmission of microorganisms from person to person or from the environment to a person (patient or health care worker) is the ability of the microbe to survive on an environmental surface.Computers are ubiquitous in the hospitals and have been shown to be contaminated with potentially pathogenic microorganism. The aim of the study was to determine microbial contamination on computer keyboards and the effectiveness of a disinfecting technique (DT).

Methods: A matched cross-over study, involving an overall of 50 computer keyboards, was conducted in two hospitals, located in Semnan city before and after the use of an innovative DT consisting of a malleable-elastic compound, containing ethanol, which adheres to surfaces, removes dirt and disinfects. Total bacterial count was evaluated and several types of bacteria and fungi were researched pre- and post-use of the DT.

Results: Before use of DT all computer keyboards were contamination with at least one bacteria especially Clostridium difficile. The DT was effective in disinfecting the keyboards. In fact, Colony-Forming Units (CFUs) decreased to zero in most comparisons before and after use of DT All the comparisons showed significant differences ($p<0.001$) after the DT.

Conclusion: It is recommended that one washes their hands before and after using a keyboard because Hand hygiene is required to prevention of contamination spread.The data suggest that microbial contamination of keyboards. These objects can be a vehicle for Health Care-Associated Infections HAIs and their disinfection should not be neglected. The DT showed to be appropriate for the disinfecting purpose.

Keywords: Healthcare-related infection, Computer Keyboard, Semnan hospitals

P949: **Improving expression and purification of C-terminal fragment of the passenger domain of Hap protein from Nontypeable Haemophilus influenzae in a highly optimized E. coli expression system**

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Background and Aim: Nontypeable Haemophilus influenzae (NTHi) is a common cause of respiratory tract disease and initiates infection by colonization in nasopharynx. The H. influenzae Hap adhesin is an auto transporter protein that promotes initial interaction with human epithelial cells. Hap protein contains a 110 kDa internal passenger domain called “HapS” and a 45 kDa C-terminal translocator domain called “Hapβ”. Hap adhesive activity has been recently referred to its Cell Binding Domain (CBD) which resides within the 311 C-terminal residues of the internal passenger domain of the protein and immunization with this CBD protein has shown to prevent bacterial nasopharynx colonization in animal models.

Methods: To provide enough amounts of pure HapS protein for vaccine studies, herein, we sought to develop a highly optimized system to overexpress and purify the protein in large quantities. To this end, pET24a-cbd plasmid harboring cbd sequence from NTHi ATCC49766 was constructed and its expression was optimized by testing various expression parameters such as growth media, induction temperature, IPTG inducer concentration, induction stage and duration. SDS-PAGE and Western-blotting were used for protein analysis and confirmation and eventually the expressed protein was easily purified via immobilized metal affinity chromatography (IMAC) using Ni-NTA columns.

Results: The highest expression level of target protein was achieved when CBD expressing E. coli BL21 (DE3) cells were grown at 37?C in 2xTY medium with 1.0 mM IPTG at mid-log phase (OD600nm equal to 0.6) for 5 hrs. Amino acid sequence alignment of expressed CBD protein with 3 previously published CBD amino acid sequences showed more than %97 identity and antigenicity plot analysis further revealed 9 antigenic domains that appeared to be well conserved among different analyzed CBD sequences.

Conclusion: Due to the presence of high similarity among CBD from NTHi ATCC49766 and other NTHi strains, CBD protein expressed here sounds to be theoretically ideal as a universal candidate for being used in vaccine studies against NTHi strains of various geographical areas. Further investigations to corroborate the potency of this protein as a vaccine candidate are under process.

Keywords: Nontypeable Haemophilus influenzae, Cell Binding Domain of HapS, optimization, E. coli BL21 (DE3), recombinant expression

P950: **Colicin typing and Distribution of multiple colicin genes in Escherichia coli in Yasuj, Iran**

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Background and Aim: Colicins are by far the best-characterized group of bacteriocins. They are produced by, and actived against, and others members of the Enterobacteriaceae family .Colicins are thought to be important mediators of intra- and interspecific interactions, and are a significant factor in maintaining microbial diversity.The aim of this study was to investigate the frequency of colicin gene types in commensalEscherichia coli strains using aPCR-based method.

Methods: This study was performed as Cross-sectional on the collection of 120 faecal isolates of Escherichia coli from children without bacterial gut infections in Yasuj which were collected over a period of six months from February to July 2012. Initially, Biochemical test being Voges-Proskauer negative, citrate negative, indole positive and methyl red positive were used to confirm the isolates as E. coli. Identification of colicin types (V, A, N, S4, B,D, D157, E1-E9, Ia, Ib, 5, 10, K, M, U and Ycolicins) was performed using nine pairs specific primers. Genotypes of all strains were verified by PCR.

Results: Of all the strains examined, 85 colicinogenicstrains (70.8%) were identified. all nine different colicins were detected. Of the 85 colicinogenic strains, 34.11% produced one type of colicin, 45.89% produced two , 20.0% produced three or more type of colicin. Strains with more than one gene encoding the colicin showed a high frequency. One strain was found to produce six different colicin. As a result the most detected gene was IaIb and the least detected gene was A.N.S4. Colicin V and Ia, Ib were found to co-occur in a strain more often than was expected by chance.

Conclusion: The results revealed that, a high percentage of bacteria isolated from feces of healthy children were generally colicinogenic strains and The presence of colicin in the gastrointestinal tract was an important factor to inhibit other pathogenic E. coli strains. The acquisition of multiple colicins by a cell confers a selective advantage to the cell because harbouring multiple colicins exploiting different surface receptors may slow the evolution of resistance in populations where the dominant colicinogenic strains produces multiple colicins compared to populations where the dominant producer encodes a single colicin.

Keywords: colicin typing, Escherichia coli , iran

P951: **Frequency of antimicrobial resistance genes among different bacterial isolates from human bile samples in patients with biliary diseases**

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Background and Aim: There are some direct associations between resistance phenotypes of bacterial whom colonizing biliary tract and their tolerance to bile products. This study was aimed to analyze frequency of main resistance genes among different bacterial isolates from human bile samples.

Methods: Antimicrobial susceptibility of each bacterial isolate to antibiotics related to β-lactams, aminoglycosides and quinolone families was assessed on Mueller–Hinton agar medium according to the CLSI guidelines (CLSI, 2011). For detection of resistance genes, total DNA from each bacterial isolates was extracted by boiling method. Detection of genes implicated in the resistance to β-lactams (blaTEM, blaCTX-M and blaSHV), aminoglycosides (aminoglycoside transferas [aadA aadB, strA and strB]) and quinolones (qnrA, qnrB, qnrC,qnrD and qnrS) were performed by polymerase chain reaction using specific primer pairs.

Results: Resistance to 3 rd and 4rd generation cephalosporins (20 up to 100%), aminoglycosides (25 up to 36.36%) and quinolones (10 up to 86%) or fluroquinolones (10 up to 100%) was detected among the isolates. While E. coli, Acinetobacter spp. and P. aeruginosa isolates showed highest ranges of resistance phenotypes, isolates related to K. pneumonia genuse showed the lowest resistance range to quinolones and ?-lactams. The blaCTX and blaTEM genes were found among 55.55% and 55.55 % of E. coli, 27.27% and 100% of P. aeruginosa, 0% and 66.66% of Acinetobacter spp., 100% and 100% of K. pneumoniae isolates, respectively. A total of 27.27% of P. aeruginosa, 100% of K. pneumoniae and 55.55 % of E. coli isolates contained both genes (blaCTX and blaTEM). None of the isolates were positive for blaSHV genes. The aadA and aadB genes conferring resistance to aminoglycosides were present in 25% and 25% of Acinetobacter spp. isolates, respectively. However, strA and strB genes conferring resistance to streptomycin were present in 22.73% of E. coli and 25% of Acinetobacter spp. strains. The qnr A, B, C,D and S were not found in any of the 25 quinolone-resistant bacteria tested.

Conclusion: Result of this study totally confirmed high frequency of main resistance genes related to different antimicrobial classes among the bacterial isolates from bile samples. Simultaneous colonization of biliary tract with these bacteria make their eradication difficult . Selection pressure of bile products for establishment of these bacteria is of major interest that may enable their life-long colonization in this niche.

Keywords: antimicrobial resistance, human bile, biliary diseases, Qnr, β-lactams, aad

P952: **In vitro activity of six plant extracts against biofilm formation and biofilm disruption of Staphylococcus aureus**

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Background and Aim: Microbial adhesion to surfaces and the consequent biofilm formation by Staphylococcus aureus is a serious trouble in the medical industries.This biofilm is global challenge for human. Bacteria embedded in biofilms are far more difficult to eradicate than planktonic infections. Therefore, the formation of biofilms greatly limits the efficacy of treatment with antibiotics.As a result,plants remain the most common source of antimicrobial agents.The aim of this study was to evaluate the effect of six extracts(ethyl acetatic and methanolic Satureja khuzistanica, ethyl acetatic and methanolic Perovskia artemisoides, methanolic Peganum harmala and Quercus brantii)on prevention of cell attachment and biofilm formation of nine clinical S.aureus isolates and S.aureus (ATCC25923).

Methods: To prevent initial cell attachment, standardised cultures of S. aureus were aliquoted into wells of a 96 well microtitre plate and extracts (with final concentration of MIC, ½MIC, ¼MIC)were added. Crystal violet staining assay was used to evaluate the ability of S.aureus strains to form biofilm in the presence of minimal inhibitory concentration of plant extracts. Disruption of pre-formed biofilms of S.aureus strains were evaluated following exposure to plant extracts at different concentration(ranging from 0.0031 to 0.4g/ml).

Results:: Most of the extracts reduced biofilm formation by at least 50%.[methanolic Peganum harmala(%56.03), methanolic Quercus brantii (%62.93), ethyl acetatic Satureja khuzistanica(%60.04), methanolic Satureja khuzistanica(%56.57), methanolic Perovskia artemisoides(75.27) , ethyl acetatic Perovskia artemisoides(%62.47)]. Disruption of preformed biofilms were observed by: methanolic Perovskia artemisoides(0.0125 g/ml), (ethyl acetatic and methanolic Satureja khuzistanica: 0.05g/ml). methanolic Peganum harmala(0.2 g/ml)and methanolic Quercus brantii(0.4g/ml)

Conclusion: Although the mechanisms of bacteriall killing and inhibition of biofilm formation are not fully understood,data from this investigation indicated a potential application for plant extracts as an adjuvant therapeutic agent for the prevention of biofilm-related infections.

Keywords: adherence, biofilm, plant extracts, minimum inhibitory concentration, Staphylococcus aureus

P953: **The rate of inducible clindamycin resistance and susceptibilities to other antimicrobial agents among Staphylococcal isolates from clinical samples in Ali ebne Hospital in Rafsanjan, Iran 2012**

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Background and Aim: Staphylococcus aureus and coagulase–negative Staphylococci (CNS) are recognized as causing nosocomial and community-acquired infections around the world. The resistance rate of these strains to various antimicrobial agents is an increasing problem. The development of resistance to Macrolide-Lincosamide-StreptotograminB (MLSB) family of antibiotics has limited the use of these antibiotics to treat of staphylococcal infections. In this present study, we investigated the prevalence of erythromycin-induced clindamycin resistance in both isolated methicillin-resistant staphylococci (MRSA) and methicillin-susceptible staphylococci (MSSA) strains in our hospital during 2012. The resistance rate of isolates to different antimicrobial agents is evaluated too

Methods: This study included 174 non duplicated isolates of staphylococci collected from clinical samples in our hospital over a time period of nine months. The isolates were first identified as staphylococcus Sp and then antimicrobial susceptibility patterns of them were tested for penicillin , cephalexin , cloxacillin , cefteriaxone , ciprofloxacin , trimethoprim-sulfamethoxazol, doxycilin , clindamycin , erythromycin ,tetracycline, gentamycin , rifampin, vancomycin , teicoplanin, Linezolid , fusidic acid and mupirocin using the Kirby-Bauer disk diffusion method. inorder to finding the presence of inducible clindamycin resistance among collected staphylococci, Double-disk diffusion test (D test) was performed for each isolate that were erythromycin resistant and clindamycin sensitive according to Clinical and Laboratory Standard Institute (CLSI) method.

Results: Of the 174 staphylococcus isolates, 65.66% were susceptible to CL, 28.73% had constitutive and 5.74% had inducible resistance to Cl. The frequencies of constitutive and inducible resistance for CL in MRSA were 55.63% and 7.89% respectively. however in MSSA isolates the frequency of the constitutive and inducible phenotype were 12.24% and 4% respectively. statistical analysis revealed the inducible resistance in MRSA isolates to be more frequent than that in MSSA isolates. The antibiotic susceptibility patterns of MRSA isolates also differed and revealed the highest resistance rate for erythromycin (73.68%), tetracycline (65.78%), cefteriaxone (63.15%), cloxacillin (57.89%) and clindamycin (52.63). None of the MRSA strains were linezolid and vancomycin resistant.

Conclusion: The study results revealed that inducible clindamycin resistance should be determined in all MRSA isolates and also staphylococcus strains resistant to erythromycin and susceptible to clindamycin by using D-Test.

Keywords: Staphylococcus, clindamycin, constitutive resistance, inducible resistance, Iran

P954: **detection of Mycobacterium tuberculosis genotypes of Beijing and non-Beijing by Melting curve analyze**

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Background and Aim: Tuberculosis is one of the most important infectiousdiseases in the world today.Rapid diagnosis of drug resistant Mycobacterium tuberculosis(MTB) is critical to starting of an appropriate treatment and preventing of more spread drug resistant MTB strains. Due to association of Beijing genotypewith drug resistance in MTB, we developed a rapid and non-culture method for detection Beijing and non-Beijing MTB in clinical samples

Methods: We modified Taqman Real time PCR for detection Beijing and non-Beijing genotypes of Mycobacterium tuberculosis to a free probe method in presence of a single dye together with amelting curve analysis. We then performed a blinded screening with both methods on 165 septum samples from treated tuberculosis patients.

Results: We were obtained the same results by both methods. Of the 33 patients, 30 samples were Beijing genotype and 135 were non-Beijing genotype.In free probe method, we were clearly identified a melting peak at 81°C corresponds to non-Beijing and a melting peak at 88°C corresponds to Beijing genotype

Conclusion: DNA melting curve analysis is a simple and efficient method for the specific detection of amplified products and greatly reduces the cost molecular detection

Keywords: Beijing genotype,Mycobacterium tuberculosis,melting curve analysis

P955: **Antibiotic resistance patterns and the prevalence of Carbapenemases enzymes among Multidrug Resistant Acinetobacter baumannii isolated from clinical specimens in hospitals of Tehran-Iran**

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Background and Aim: Multidrug resistant Acinetobacter baumannii is an increasingly significant causes of nosocomial infection worldwide. One of the drugs that have been used extensively to treat these bacterial infections is Betalactam family. In other hand, these bacteria produce the Betalactamase enzymes against to that drugs.Carbapenemases found in A. baumannii so far belong to either the OXA class D family of serine beta-lactamases or IMP/VIM class B family of metallo-beta-lactamases.Furthermore, the KPC is another carbapenemases enzyme that belongs to class A family of Ambler classification.The purpose of current study was to define the antibiotic susceptibility patterns and detect the prevalence of producing strains of extended-spectrum β-lactamase (ESBL) in A. baumannii isolates which had been isolated from clinical samples with combined disk test and determine the distribution of bla OXA-23 , bla IMP and bla KPC genes in A. baumannii which had been isolated from clinical samples with PCR assay.

Methods: This study was conducted in 3 major hospitals (Imam Khomeini, Milad and Baqiyatallah) in Tehran on 400 clinical samples during one year. After identification of isolates in species level using cultural and biochemical methods, in order to determine sensitivity of 100 isolates of A. baumannii to 9 antibiotics, the susceptibility tests were carried out according to CLSI guidelines using disk diffusion method.Furthermore, to identify of producing strains of ESBL was applied phenotypic method of combined disk, and then for detecting bla OXA-23, bla IMP, bla KPC genes used PCR assay.

Results: In this survey, 100 A. baumannii strains, 30 A. lwoffii strains and other Acinetobacter species were isolated from patients. The majority of isolates were from blood specimens. Isolates of A.baumannii revealed the highest resistance to amikacin, imipenem, piperacillin - tazobactam, meropenem, gentamicin, tobramycina and tetracycline, respectively. Ampicillin - sulbactam and polymyxin B considered as effective drugs in this study. Multidrug resistance in these strains was 70%. According to the results of combined disk test, 20% of total samples were demonstrated to be ESBL positive. The PCR results showed that 37%, 19% and 12% of isolates had bla OXA-23, bla IMP, bla KPC genes respectively, which most of them had been isolated from patients who were hospitalized in the ICU.

Conclusion: Regarding to produce of ESBL and existence of bla OXA-23, bla IMP, bla KPC genes in this bacterium and possibility of transformation of these genes to the other bacteria, reconsideration in antibiotics consumption patterns as well as more attention to nosocomial infections control criteria are inevitable.

Keywords: Acinetobacter baumannii,Extended-spectrum β-Lactamase (ESBL) , Combined disk, Carbapenemase.

P956: **Nosocomial Transmission of Cefotaximases enzymes Producing Acinetobacter baumannii in hospitals of Tehran by PCR method**

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Background and Aim: The majority of clinical A. baumannii isolates are highly resistant to a variety of antibiotics, including betalactams, which are currently the drugs of choice in the treatment of the severe infections caused by this organism. Resistance in A. baumannii is associated with a variety of combined mechanisms that one of them is the acquisition and production of beta-lactamases CTX (blaCTX) which belongs to class A of Ambler and 2be of Bush classification. In this study, we assessed the prevalence of bla CTX-M gene in clinical A. baumannii isolates in three hospitals located in Tehran by PCR method.

Methods: this study was performed on 400 isolates of Acinetobacter which were isolated from patients in 3 major hospitals (Imam Khomeini, Milad and Baqiyatallah) in Tehran during 6 months. After the identification of strains of A. baumannii using culture and biochemical methods, test sensitivity to 4 antibiotics on 100 isolate of A. baumannii by using the disk diffusion method on CLIS (Clinical and laboratory standards institute) was performed. Also MIC (Minimum inhibitory concentrations) was determined for cefepime and ceftazidime and then we used PCR assay for detecting bla CTX-M gene .

Results: In this survey, 100 A. baumannii strains, 30 A. lwoffii strains and other Acinetobacter species were isolated from patients, The majority of isolates were from blood specimens. Isolates of A.baumannii revealed the highest resistance to cefepime, ceftriaxone, cefotaxime and ceftazidime, respectively. Minimum inhibitory concentrations of ceftazidime in 84% and cefepime in 91% samples were above 128 g/ml. The PCR results revealed that 19% of isolates harbored bla CTX-M genes.

Conclusion: A. baumannii strains harboring CTX-M are currently widely distributed throughout the Tehran - Iran. Only 19% of our isolated carried blaCTX-M gene, so other mechanisms in bacteria such as secretory pumps and changes in purine cause resistance. The rapid identification and detection of these strains is an important role in preventing their spread.

Keywords: Acinetobacter baumannii, ESBL , Cefotaximase

P957: **Prevalence, phenotypic and genetic characterization of Carbapenemase and ESBLs producing Gram-negative bacteria (GNB) isolated from patients with cystic fibrosis in Tehran hospitals.**

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Background and Aim: Cystic fibrosis (CF) is an autosomal recessive genetic disorder in white populations caused by mutation in a gene that encodes cystic fibrosis transmembrane conductance regulator (CFTR) protein. Since frequent respiratory tract infections are the major problem in patients with CF, obligation to identify the causative bacteria and determining their antibiotic resistance pattern is crucial. The purpose of this project was to detect Gram-negative bacteria isolated from sputa of cystic fibrosis patients and to determine their antibiotic resistance pattern.

Methods: The sputum of 52 CF patients, treated as inpatients at hospitals in Tehran, was obtained between November 2011 and June 2012. Samples cultured in selective and nonselective media and Gram-negative bacteria recognized by biochemical tests. Antimicrobial susceptibility testing to cephalosporins, aminoglycosides and carbapenems was performed by disk diffusion method then MICs of them were measured by using broth microdilution method . For phenotypic detection of carbapenemase and ESBLs production, the Modified Hodge test, EDTA disk synergy test and the combined disk methods were performed. Subsequently, the gene encoding the extended spectrum beta-lactamases (blaPER, blaCTX-M) and carbapenemases(blaIMP-1 , blaGES, blaKPC, blaNDM, blaVIM-1 ,blaVIM-2, blaSPM, blaSIM) in Gram negative bacteria were targeted among the resistant isolates by using PCR. PFGE was used to determine any genetic relationship among the Pseudomonas aeruginosa isolated from these patients.

Results: Fifty six Gram negative bacteria isolated from 52 sputum samples include Pseudomonas aeruginosa, Klebsiella ozaenae, Alcaligenes xylosoxidans, Achromobacter denitrificans, Klebsiella pneumonia , chryseobacterum daecheongense and Stenotrophomonas maltophilia. The rates of resistance to different antibitics were as follows: cefixime (%80), ceftriaxone (%43), ceftazidime (%45) and meropenem (%7). Twenty three strains showed the highest rate of resistance to tested antibiotics. Of these 23 isolates, 17 were resistant to carbapenems (4 showed MBL activities) and 16 were positive for ESBL activities.The prevalence of genes encoding the ESBLs and Carbapenemases among the phenotypically positive strains were as follows: blaCTX-M (19), blaPER(1), blaIMP-1(2), blaVIM-1(2)and blaVIM-2 (3) genes respectively. No other genes were detected. PFGE analysis revealed 8 genotypes. 6 isolates had mutually 3 similar patterns.

Conclusion: This study showed the existence of important ESBLs and Carbapenemases genes among the Gram-negative bacteria isolated from patients with CF. Continuous surveillance of ESBLs and Carbapenemases, also identification of their types, in bacteria isolated from these patients have an important clinical impact, since it can often provide valuable information for effective infection control measures and for the choice of appropriate antimicrobial therapy.

Keywords: cystic fibrosis, GNB (Gram Negative Bacteria), PFGE, Carbapenemase, PCR.

P958: **DNA identification of Helicobacter pylori in gallstone**

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Background and Aim: bacterial infections have been accepted as a gallstone causing agent. In the sense that, these infections have occurred between people who have previous backgrounds of Hypercholesterolemia and not consistent dieting incidents. The aim of this study is to determine Helicobacter pylori in a handful of gallstones among patients.

Methods: the gallstone samples from 36 patients with gallbladder disease were admitted into Shahid Rajayi hospital in 1391 and were immediately transferred to the laboratory under sterile conditions. The central part of the gallstone was smashed and cultured in Pristine supplemental condition in the initial enrichment and after 72 hours with turbidity observed,DNA was extracted by using a kit. Using the PCR technique and implementing specific primers, confirmed the presence of bacteria.

Results: Patients in terms of sex, age and type of gallstones Were examined, in particular among patients, 30(83/33%) Women and 6(16/66%) male. Patients with gallstones by the age of 55, range 22-82 years of age group or under 30 years, 4 (11/11%), 30 to 40 years, 5 (13/88%) and above 40 years 27 patients (75%). The samples of rock of the 14 stones Cholesterol Net (38/88%), 7 stones Cholesterol Mixed (19/44%) and 15 pigmented stone(41/66%) were diagnosed Of 36 cases of gallstones in 3 cases (33/8%) were positive for the presence of Helicobacter pylori.All of them were in the age group above 40 years. However, no significant relationship was found between the rocks and the presence of bacteria DNA.

Conclusion: The results of this study indicate the presence of Helicobacter pylori DNA by using PCR techniques and specific primers.Therefore, further studies will investigate the role of H.pylori in Gallstone formation.

Keywords: DNA, H.pylori, PCR , Gallstone

P959: **The study of relation between biofilm formation of Staphylococcus aureus and methicillin resistant**

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Background and Aim: Methicillin Resistant Staphylococcus aureus (MRSA) is one of the main factors of nosocomial infections in the world. Biofilm formation which plays an important role in bacteria attachment , is one of the important virulent factor of these bacteria. The aim of this research is the study of relation between the Biofilm formation and Methicillin resistance in Staphylococcus aureus.

Methods: In this study, 65 samples were prepare from Shahrood's Hospitals and Staphylococcus aureus ATCC 25923 was used as control. In order to study of Methicillin Resistant Staphylococcus aureus, Kirby – Bauer method was used.Investigation of biofilm formation was done by 96 microtiter plate method.

Results: In this study , among 45 methicillin-resistant St.aureus , 20 samples showed resistant to methicillin which are selected as the best samples for biofilm formation test. Among these strains, 66.6% formed strong biofilm, while 33.3% showed moderate ability to form biofilm and attachment.

Conclusion: According to this study, there is a direct relationship between biofilm formation and antibiotic resistance and we also require a wide range of antibiotics for the infection in S.aureus which has formed biofilm.

Keywords: Methicillin Resistant Staphylococcus aureus (MRSA), biofilm, Antibiotic resistance.

P960: **Immunogenicity Evaluation of E. coli O157: H7 Detoxified Lipopolysaccharide - Tetanus Toxoid (D-LPS-TT) Conjugate and D-LPS against E. coli O157: H7 Infections**

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Background and Aim: E. coli O157: H7 is one of the main factors leading to food toxicity and diseases such as diarrhea, bleeding colitis, uremic hemolytic syndrome, thrombocytopenic purpura and even death in man. E. coli O157: H7 LPS is a key factor in virulence. The aim of this study was immunogenicity evaluation of E. coli O157: H7 D-LPS-TT conjugate and D-LPS against E. coli O157: H7 infections in mice model.

Methods: LPS from E. coli O157: H7 was extracted with modified hot phenol method and detoxified by NaOH. For conjugation to tetanus toxoid, was linked ADH as a spacer molecule and EDAC as a linker. D-LPS-TT was purification by gel filtration. D-LPS-TT and D-LPS were injected intraperitoneally to animal model. Vaccination was performed by 3 doses. Then serum samples were collected and antibodies response against LPS was measured by ELISA method for total IgG, IgM, IgA, IgG1, IgG2a, IgG2b and IgG3.

Results: The results of anti-LPS inductions for total IgG, IgM, IgA, IgG1, IgG2a, IgG2b and IgG3 were observed D-LPS-TT>D-LPS. These results indicated that LPS from E. coli O157: H7 increases anti-LPS antibodies in conjugate form with tetanus toxoid and can be appropriate effective conjugate.

Conclusion: D-LPS-TT showed significance increasing in all types of antibodies titers concentration against LPS in versus D-LPS, thus conjugate form of E. coli O157: H7, will be useful tools for elucidating E. coli O157: H7 infections.

Keywords: E. coli O157: H7, D-LPS-TT, D-LPS, Conjugate, Immunogenicity

P961: **Study of Antigenic Structure Stability of E. coli O157: H7 Detoxified Lipopolysaccharide-Tetanus Toxoid (D-LPS-TT) Conjugate**

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Background and Aim: Both large molecular weight polysaccharides and small, otherwise non-immunogenic oligosaccharides can be converted to thymus-dependent (TD) antigens by conjugation to a carrier protein. The aim of present study is antigenic structure stability evaluation of E. coli O157: H7 detoxified LPS in conjugate form.

Methods: E. coli O157: H7 were isolated from bloody diarrhea patients suffering to zanjan city. LPS was isolated from E. coli O157: H7 with modified hot phenol method. To improve immunogenicity, the purified antigen was coupled to tetanus toxoid with adipic acid dihydrazaid as a spacer and EDAC as a linker. Finally the reaction mixture was passed through a Sepharose CL-2B column and outcome fractions was spected at two wavelength 280 and 210 nm, the fractions with high absorbed joined with together. The resulting conjugate was non-pyrogen and non-toxic.

Results: Investigation of apparent fraction at 280 and 210 nm according to conforming, it shows conjugate was composed of TT and large-size D-LPS polymer at a ratio of about 3: 1. This conjugate was non-toxic and non-pyrogenic.

Conclusion: The results showed extraction LPS was active and after conjugation it remained on the true forms with protective all epitopes and we can use it for injection to animal model and study induce antibody.

Keywords: E. coli O157: H7, LPS, Tetanus toxoid, Conjugate

P962: **isolation, identification and characterization of Campylobacter spp. and Arcobacter spp. from Kor River**

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Background and Aim: Campylobacter spp. are important causative agent of gastric infection worldwide, and contaminated water and foods are the major transmission factors of this bacterium to human. The major purpose of this study was isolation, identification and characterization of Campylobacter spp. and Arcobacter spp. from the samples obtained from Kor River.

Methods: 200 water samples were collected throughout four seasons. Campylobacter spp. and Arcobacter spp. were isolated using standard methods and were identified by phenotyping tests. Finally, the identification of these strains was verified by PCR method.

Results: Folowing phenotyping tests and their confirmation with molecular technique, totally seven Campylobacter jejuni strains and 14 Arcobacter butzelri strains were identified. Based on the results, the prevalence of this bacterium in the coastal waters of the Caspian sea were evaluated as 2.66 and 5.32 percent.

Conclusion: It is the first time that Campylobacter jejuni and Arcobacter butzelri were isolated from kor river. The epidemiologic studies regarding to the ways of their entrance in an environment and their maintenance in the habitat assist activists to control the water qualification and prevention from distribution of infections.

Keywords: Campylobacter, Arcobacter, Isolation, PCR

P963: **First report of class 1 integron identified in Staphylococcus aureus isolated from clinical samples in Iran**

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Background and Aim: Staphylococcus aureus is a major pathogen in causing disease and death in the world. In recent years introduced a variety of antibiotics with activity against these organisms due to acquired drug resistance and Severe infections in burns, In different parts of the body Has created. Indiscriminate use of antibiotics to treat bacterial infections caused by resistant strains are selected. Unfortunately, the risk of transmission of resistance genes by susceptible strains of the bacteria are growing. Due to the increasing prevalence of resistance to many commonly used antibiotics, treatment is very difficult to fight against it. According to the World reports, Staphylococcus aureus strains resistant to antimicrobial agents is increasing. One reason for this increased carry and transfer of antibiotic resistance genes on the integron structure. Integrons are gene sets that can, mobile genetic elements called packets gene (gene casset) can the moving gene casset and In areas 3/ and 5/ have fixed parts.The aim of this study was to investigate the presence of class 1 integron in S. aureus strains isolated from clinical samples in Tehran.

Methods: In this study, 200 isolates of S. aureus from patients admitted to the baghiyatollah hospital in Tehran was isolated and studied. This strain was confirmed using biochemical and serological tests.. And strains containing class 1 integron with the technique of polymerase chain reaction (PCR) using specific primers were identified.

Results: The findings of this study whith the PCR method showed, 200 isolates obtained from only 1% strain (2 strain) exhibited class 1 integron gene.

Conclusion: The results of this study showed, Given the increasing prevalence of antibiotic-resistant strains of Staphylococcus aureus and the One reason for the presence of antibiotic resistance genes are integron. Use of antibiotics to identify genes in the integron is desirable. It must take to prevent the spread of antibiotic resistance genes using integron continued.

Keywords: Staphylococcus aureus, integron class 1, PCR

P964: **In vitro activity of photodynamic inactivation and azithromycin against upper respiratory tract isolates grown as biofilm**

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Background and Aim: Biofilms have been found to be involved in a wide variety of microbial infections in the body, by one estimate 80% of all infections. Bacteria living in a biofilm usually have significantly different properties from free-floating bacteria of the same species, as the dense and protected environment of the film allows them to cooperate and interact in various ways. One benefit of this environment is increased resistance to detergents and antibiotics, as the dense extracellular matrix and the outer layer of cells protect the interior of the community. In the head and neck area, biofilms are a major etiologic factor in periodontitis, wound infections, oral candidiasis, and sinus and ear infections. For the past several decades, photodynamic inactivation (PDI) has been reported in the literature to be effective in eradicating various microorganisms. PDI involves killing of organisms by light in the presence of a photosensitizing agent. This study aimed to determine if PDI was effective in the treatment of upper respiratory tract isolates growing as biofilms and whether a synergistic effect was evident if PDI was used in combination with azithromycin.

Methods:: The susceptibility of biofilm culture of isolates (Staphylococcus aureus, Staphylococcus saprophyticus, Staphylococcus haemolyticus, Klebsiella pneumoniae and Candida albicans) to methylene blue/toluidine blue O- mediated PDI (MB/TBO-PDI) was determined alone and in combination with azithromycin (1/2 MIC).

Results: When isolates were grown in biofilm, PDI treatment alone was not bactericidal. When PDI was combined with antibiotic treatment, bactericidal activity was apparent. TBO at 200µM, 27 J/cm2, in combination with azithromycin (1/2 MIC), exhibited 2.3 log10 killing for S. aureus, 3.8 log10 for S. saprophyticus,3.7 log10 killing for S. haemolyticus, 2.8 log10 killing for K. pneumoniae and 4 log10 killing for C. albicans. However, MB at 200µM ,27 J/cm2 , in combination with azithromycin (1/2 MIC), exhibited 3 log10 killing for S. aureus, 4 log10 for S. saprophyticus, 4 log10 killing for S. haemolyticus, 3 log10 killing for K. pneumoniae and 4.5 log10 killing for C. albicans.

Conclusion:: PDI could be a possible treatment option in combination with azithromycin for upper respiratory tract isolates grown as biofilms. Keywords: photodynamic inactivation, biofilms, antibiotic therapy

Keywords: PDT,sub MIC Azithromycin,Upper respiratory tract infections,MB/TBO

P965: **Detection of Herpes Simplex Virus I & II in Multiple Sclerosis patients by polymerase chain reaction**

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Background and Aim: Herpes simplex virus type-1 (HSV-1) and type-2 (HSV-2) are widespread human viruses frequently detected in specific tumors and immunosuppressed individuals. On the other hand one of the most important immune depended diseases is Multiple sclerosis (MS) that has several unknown reasons and HSV 1&2 possibly can be one of them. The purpose of this study is detection of Herpes Simplex Virus in Multiple Sclerosis patients by polymerase chain reaction (PCR).

Methods: In this study two sets specific primer for detection of Herpes simplex virus 1 & 2 have been selected. PCR has been optimized and sensitivity and specificity of tests carried out. In addition Amplicon was cloned and sequenced by Dideoxy chain termination. By using DNG method the DNA of specimens were been extracted. This study has been performed on 100 samples which obtained from patients suffering MS in a hospital and 100 healthy samples.

Results: The products of optimized PCR with 271bp and 231 bp length repectively for HSV1 and HSV2, correctly amplified and observed on electrophorese gel. The two tests had a high sensitivity and specificity level. From 100 studied samples with HSV-1& II primers, in 6 samples, HSV-1 has been detected but healthy ones have not any. From 100 samples of patient and 100 healthy with HSV-2 primer we haven't found any HSV-2 viruses.

Conclusion: Our results showed that HSVI found in a percentage of MS patients and didn’t observe in any healthy controls. These data indicate that HSVI reactivate in MS patients and probably play a role in MS.

Keywords: Herpes simplex virus I & II, PCR, Multiple sclerosis

P966: **Design, cloning and expression of TGFαL3-SEB fusion protein as an anti tumor candidate in cancer immunotherapy**

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Background and Aim: Activation of the immune system is one of several promising therapeutic methods for controlling cancer progression in patients. Tumor cells often avoid presenting their own antigens to T cells. Superantigens (SAgs) are bacterial and viral proteins that can activate a large number of T cells irrespective of their antigen specificity, resulting in a massive release of cytokines from T cells and monocytes. They increase the antitumor activity of the immune system and prevent tumor growth and metastasis. Recent attempts have done to specifically target superantigens towards tumors, subsequently monoclonal antibodies and tumor-related ligands have been employed as targeting molecules of superantigen for the preclinical treatment of a variety of tumors. Generally, the targeted antigen or receptor should have a high density on the surface of the target cells so the epidermal growth factor receptor (EGFR) was chosen as a suitable receptor for the design of ligand-targeted therapeutics in cancer immunotherapy, Overexpression of EGFR protein has been described in various human carcinomas. Moreover, human transforming growth factor alpha (hTGFa) is a native ligand co-overexpressed with its receptor EGFR in many human tumors. Here, we designed the chimeric construct containing the third loop of transforming growth factor alpha and staphylococcal enterotoxin B (TGFαL3-SEB) and express in E.coli as a new antitumor candidate.

Methods: After screening 300 Staphylococcus aureus isolated from clinical samples for seb gene presence, one positive strain was found. The seb gene was amplified by using specific primers and cloned into the pET28a vector (pET28a:: seb), after that the sequence of tgfα-l3 gene with linker was synthesized and cloned before seb gene to produce pET28a:: tgfαl3-seb recombinant vector construct that was confirmed by sequencing. The pET28a:: tgfαl3-seb recombinant plasmid was transformed into E. coli strain BL21 (DE3) and the culture was grown to an optical density (600 nm) of 0.5–0.7. Expression of the chimeric sequence was achieved by the addition of 0.5mM IP. SDS-PAGE analysis was performed to confirm the recombinant fusion protein (~31 kDa). The recombinant protein was purified using nickel nitrilotriacetic acid (Nii Acetic acid (Nias purified using)). Finally Western blot analysis was done to confirm the presence of 6x His-tagged TGFαL3-SEB fusion protein.

Results: Restriction digestion and sequence analysis confirmed the correctness of length, position and orientation of inserted fusion genes. TGF?L3-SEB was expressed and purified; SDS-PAGE and western blot analysis confirmed the presence of TGF?L3-SEB fusion protein.

Conclusion: In this study, we designed a new construction containing SEB and TGFαL3 the premilary of experiment was done and this recombinant protein could be usable as an anti tumor candidate in future studies.

Keywords: Cancer Immunotherapy, Cloning, Epidermal growth factor receptor, Staphylococcal enterotoxin type B

P967: **Investigation of the Anti-Helicobacter pylori antibodies Produced in Laboratory animal for application purposes**

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Background and Aim: H. pylori infects more than one half of the world population. The spectrum of clinical disease associated with H. pylori infection is wide and include chronic gastritis, gastric and duodenum ulcer, gastric adenocarcinoma and MALT lymphoma. H. pylori infection can be detected by various diagnostic methods including invasive and noninvasive however, the non-invasive methods have some advantages. The aim of present study was to identify the conserved immunogenic antigen among the outer membrane proteins (OMPs) of H. pylori.

Methods: The sarcosine-insoluble OM fractions of H. pylori strains were prepared and their SDS-PAGE profiles were compared. Two strains with more conserved profiles were selected for immunization of rabbits. Then 100 µg of the proteins were injected to white female rabbit and serum sample was collected after third intermittence. Mono-specific-anti H. pylori antibodies were obtained using affinity chromatography and the titers of antibodies were measured by ELISA. The antibody titers were compared before and after purification of antiserums. The individual antibodies developed against conserved OMPS were detected by Western Blotting.

Results: Five conserved OMPs were observed among various H. pylori strains. The titer of antiserum determined by ELISA was 1/2000 before purification. Lower titers were obtained after affinity chromatography suggesting presence of cross-reacting antibodies in rabbit antiserum. The results of western blotting indicated presence of four specific OMPs with molecular weights of 21, 25-26- and 34-KDs.

Conclusion: The result of this work has revealed that among OMPs of H. pylori, at least four conserved immunogenic proteins can be detected in various strains. Furthermore, difference between the titers of antiserums before and after purification would, explains that the cross-reacting antibodies in rabbit may increase the rate of false positive results in ELISA method.

Keywords: Helicobacter pylori , ELISA , Affinity Chromatography , Western blotting

P968: **s Blood′Bacteriological and molecular analysis in rheumatoid arthritis patient**

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Background and Aim: Rheumatoid arthritis (RA) is the most common chronic inflammatory disease of unknown etiology. The roles of bacterial super antigens as effective agent were considered. Therefore, the aim of this study was to the Bacteriological investigation and the profile of laboratory s blood samples.′characteristic of rheumatoid arthritis patient

Methods: During the 9 months cross-sectional study of patients whom referred to rheumatology clinic and with an initial diagnosis of rheumatoid arthritis, 80 blood samples were collected in a volume of 15ml. 10ml of them was inoculate to Castaneda medium, and remained 5 ml, were collected in the test tubes for obtain serum and plasma. Therefore, in addition to bacteriological culture and molecular PCR assay, the other tests such as WBC, PLT, CRP, ESR, CR, RF, AST, ALT, ALP, and Anti-CCP was carried out. Also, after incubation by using colony characteristics, Gram stain, catalase, oxidase, motility, sugar fermentation, susceptibility to bacitracin and Novobiosin, coagulase and DNase were studied.

Results: During 9 months, 80 patients with rheumatoid arthritis were included. With aseptic precautions conditions 5 to 10 ml of blood, was inoculated and incubated into each blood culture. Then, sequential sub cultures only 4 positive cases were obtained and base on the results of biochemical tests a gram positive cocci (possibly Staphylococcus intermedius) and three Gram-negative bacilli (possibly two Pseudomonas putida strains, and one P. aeruginosa strain) were detected. The result of Molecular diagnosis of enterotoxin C gene of this bacteria was negative. Also, gene extraction from the s detail′s buffy coat of these patients were assayed and it′blood results will be presented in the original paper.

Conclusion: The role of Bacterial super antigens was considered in the pathogenesis of rheumatoid arthritis, but its origin is unknown. Thus, proof of endogenous origin can provide a good model for the diagnosis and treatment of disease. The results of this study have been shown some evidence of being endogenous origin for involved super antigens.

Keywords: rheumatoid arthritis, super antigens, bacteriological culture, PCR.

P969: **Use of Nested PCR to detect of chlamydia trachomatis from spontaneous abortion women in 2012**

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Background and Aim: Spontaneous abortion (SAB), the loss of a concepts prior to 20 week, it the most common adverse outcome of pregnancy. Phenotypic, chromosomal, environmental factors may have impact on SAB occurring in pregnancy. Evidence suggests that sexually transmitted bacterial infection such as chlamydia trachomatis (CT), Mycoplasma genitalium, ureaplasma parvum and Neisseria gonorrhoeae may play role in SAB. Chlamydia trachomatis has been detected in cervical salpingeal, endometrial samples, pelvic inflammatory disease (PID), infertility, abortion and upper genital tract during pregnancy. This study was performed to diagnosis the prevalence of CT and to study the correlate parameters in aborted women who referred to Shahid Beheshti medical center during 2012.

Methods: Following removal of excess Endocervical mucus, Dacron swab (Delta lab product, Spain) was used to collect from 121 aborted women for Nested PCR .The specimen collected was placed in 1.5 ml of 0.2 M sucrose phosphate (2SP) Chlamydia transport medium prepared according to the method of Gordon et al. The PCR specimens were stored at -70 °c. 200 µl of 2SP medium used for DNA extraction with Accucurp extraction DNA kit (Bioneer,korea) and performed Nested PCR in which OMP1primers were used for amplication of outer membrane gene of CT

Results: One hundred twenty one Endocervical specimens were for PCR assay that had 17-38 years old (average 28.6 years). Analysis of PCR showed that 16 PCR positive sample (13.2%) were found to be positive in the Nested PCR with the OMP1 primers as well as the Chlamydia trachomatis .All of sixteen specimens were < 28 years old . personal history of women's had Chlamydia trachomatis indicated that 31.2% had academic education ($p<0.3$) , 81.25% being housewives ($p<0.006$) ,43.75% used natural methods for contraception ($p<0.002$) ,68.7% had upper three times intercourse in week ($p< 0.001$) , 75% had previous abortion ($p< 0.005$) and 68.9% had not history of pregnancy ($p<0.3$).

Conclusion: This study show that prevalence of Chlamydia trachomatis in Iran is high because these bacteria in couple with have no clinical symptomatic and require facilities of PCR in general laboratory. Anyway, Ministry of health is obligatory proceeding with control program to reduce Chlamydia trachomatis infection and other sexual transmitted disease.

Keywords: Chlamydia trachomatis,spontaneous abortion,Nested PCR

P970: **Determination of drug resistant pattern of pathogenic Campylobacter isolated from fecal samples of poultry**

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Background and Aim: Campylobacter are normal flora of warm blood animals and poultry. These bacteria could cause enteritis in human. Although the disease is self limited ,in severe case antibiotic therapy is recommended.

Methods: The present study antimicrobial susceptibility of pathogenic campylobacter isolated from poultry was determined using disk diffusion and minimal inhibitory concentration by E-test.To perform the investigate 37 pathogenic Campylobacter Viz., C.jejuni, C, coli and C.lari were isolated from poultry. Then resistant pattern of them against antibiotics cotrimaxazol, chloramphenicol, cefotaxim, ampicillin, ciprofloxacin, tetracycline, erythromycin, gentamicin and cephaloxin was determined.

Results: The results obtained from antimicrobial susceptibility of pathogenic Campylobacter indicated that all strains were sensitive to ciprofloxacin however; the isolates had different response to the other antibiotics. The results obtained from minimal inhibitory concentration of effective antibiotics indicated that ciprofloxacin with relatively less concentration and ampicillin and chloramphenicol with relatively high concentration inhibit growth of Campylobacter.

Conclusion: However, ciprofloxacin has been identified as best antibiotic for treatment of campylobacterosis. But antibiotic resistant Campylobacter existed in different region of our country.

Keywords: drug resistance, pathogenic Campylobacter, Antibiotics

P971: **Urinary Tract Infection prevalence and Antibiotic Resistance pattern among Persian women: March2010-March2011**

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Background and Aim: many pregnant women suffer from Urinary tract infection (UTI) during their pregnancy. UTI if untreated may lead to serious life threatening condition and morbidity in pregnant women. This study aimed to assess bacterial profile that causes urinary tract infection and their antimicrobial susceptibility pattern among pregnant women who were referred to labratories due to symptomatic UTI

Methods: A cross-sectional study was conducted in health care center of the city during March 2010-March 2011. Mid-stream urine samples were collected and inoculated into EMB and blood agar. Colony counts yielding bacterial growth of 105/ml of urine or more of pure isolates were regarded as significant bacteriuria for infection. A standard method of agar disc diffusion susceptibility testing method was used to determine susceptibility patterns of the isolates.

Results: The predominant bacterial pathogens were Escherichia coli 85.6% followed by coagulase-negative staphylococci, proteus, Klebsiella, and Enterobacter. Isolated pathogens showed high resistance to Penicillin and Ampicillin and Amoxicillin

Conclusion:: E-coli are the most uro- pathogen among pregnant women and drug resistance is increasing dramatically.our results show a map to health planner in order to control UTI among pregnant women.

Keywords: Urinary Tract Infection, Pregnant women, antibiotic resistance

P972: **Survay Adenosine deaminase (ADA) in the serum of patients with hepatitis B and comparison with healthy controls**

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Background and Aim: Adenosine deaminase (ADA) specifications (EC3.5.4.4) is an enzyme hydrolyse that breaks Adenosine and deoxiadenosine to inosine and deoxinosine and ammonia, In other words, the ADA is involved in purine metabolism. The highest levels are of adenosine deaminase activity in monocytes and lymphocytes. This enzyme has three isoenzyme ADA1 and ADA2 and is ADA1 + CP. Elevated serum ADA activity with increased serum levels of AST, ALT and imunoglobulins were reported in portal cirrhosis, hepatitis, jaundice and liver fibrosis, hepatoma and acute infective hepatitis. We aimed to investigate the activity of total ADA in serum of patients with hepatitis B And were determined molecular weight ADA1 and ADA2 isoenzymes in serum and RBC of these patients.

Methods: We were define Experiments by electrophoresis on SDS-PAGE, isozymes in serum and red blood cells. The ADA activity was assayed by the modified Ellis method with a model 912 type Automatic Analyzer (Hitachi Co. Ltd., Tokyo, Japan). Total ADA were measured in 37 patients with hepatitis B and 40 healthy controls in the age range (20-60 years).

Results: ADA1 molecular weight was estimated at about 35 KDa and ADA2 about 110 KDa. In our review Total ADA (tADA) enzyme activity is in controls and patients with hepatitis B, respectively, 13.35 ± 2.12 and 27.05 ± 7.86. Our results indicated that tADA level were higher in patients with hepatitis B than those of corresponding controls ($P < 0.05$).

Conclusion: tADA enzyme activity shows a significant increase compared to the control group in all age groups tested. Therefore, serum ADA level could be used as an indicator along with other parameters in follow up of patients with hepatitis B.

Keywords: Adenosine deaminase, Isoenzymes, hepatitis B, SDS-PAGE electrophoresis

P973: **Survy of hepatitis C RNA in peripheral blood mononuclear cells of patients with chronic hepatitis C**

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Background and Aim: HCV is a single stranded RNA virus of the flaviviridae family that replicates by its negative strand. Hepatitis C (HCV) is the leading cause of liver disease infecting. In most cases of infection (85%) the virus evades the immune system and establishes a chronic infection that may lead to cirrhosis and liver carcinoma. Liver is the main site of HCV replication; HCV RNA has been detected in circulating extra hepatic sites, such as in peripheral blood mononuclear cells (PBMC) .It has been proposed that PBMC could be the source of recurrent HCV infection. The aim of this study was to investigate the presence of HCV RNA in PBMCs of patients with chronic hepatitis C after sustained virologic response to antiviral treatment after 5years.

Methods: About 67 patients with chronic HCV infection after treatment, 20 patients with HCV infection and 20 healthy samples as control groups were analyzed in this study. Blood samples were collected in a sterile tube containing EDTA .Then buffy coat layer of cells was collected after centrifugation. RNA was extracted from plasma and PBMCs by the guanidium isothiocyonate method. The extracted RNA was amplified by RT-PCR method.

Results: None of patients had positive results for HCV RNA in serum and PBMC samples in treatment and healthy control groups. HCV RNA was detected in serum and PBMC samples of 20 patients with HCV infection.

Conclusion: All patients had clearance of HCV RNA in both serum and PBMCs after 5 years of response to antiviral treatment.

Keywords: HCV, sustained virologic response (SVR), RT-PCR, antiviral treatment T , PBMC

P974: **DETECTION OF TYPHOID CARRIERS**

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Background and Aim: Chronic carrier Salmonellosis can be spread by chronic carriers who potentially infect many individuals. There are major limitation in current diagnostic methods in detecting the " carrier " status . Perfect diagnostic test for typhoid and Typhoid carriers be quick, specific and Sensitive. The aim of this study is to review common methods of diagnosis of typhoid carriers.

Methods: This is a review study .we used scholar google web search engine, pubmed database . The key words that we searched in papers includes " Typhoid carrier " and "typhoid antigen".

Results: Gold standard method to identify chronic carriers of Salmonella Typhi is bile aspiration or duodenal secretions, but these methods are invasive and practically not possible. Bacteriological methods such as culture carriers also required several turns stool culture or a culture of bile or duodenal fluid. Various studies used different methods for detecting and diagnosing carriers of Salmonella Typhi such as urine and fecal culture, ELISA, and PCR The need for an alternative, low cost test for typhoid has also spurred the development of other serological assays including counterimmunoelectrophoresis, ELISA ,RIA and the haemagglutination assay ,Coagglutination tests have also been used for the detection of antigens in urine and serum .

Conclusion: The development of a test method using the immunochromatography strips may be appropriate for carrier diagnosis in this regard.

Keywords: Typhoid carrier , Typhoid antigen ,Typhoid carrier diagnosis

P975: **Comparison of four DNA extraction methods from gram-negative and gram-positive bacteria**

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Background and Aim: DNA extraction is the first step in the genetic engineering research and obtains a suitable protocol for the preparation of a purified DNA is essential. In this context, many manual and kit methods were provided. The aim of this study is to achieve a faster and low cost method for DNA extraction of Gram-positive and gram-negative bacteria. We compared four DNA extraction methods, phenol chloroform, using type 5 enzymes detergent laundry brand tag and boiling.

Methods: In this study, bacterial suspensions of Staphylococcus aureus, Vibrio cholerae, Klebsiella pneumoniae and Salmonella typhimurium were produced similar to McFarland 0.5 turbidity. Bacterial DNA was extracted with using four different methods and three times for each. With using spectrophotometer and gel electrophoresis were measured concentrations and quality of DNA extraction product accordingly. In order to evaluate the efficiency of DNA extraction method, PCR and REP-PCR were performed on some of housekeeping genes downstream regions.

Results: The concentration of the extracted DNA and results of PCR and REP-PCR were different between Gram-positive and Gram-negative bacteria significantly ($P< 0.05$). Efficacy of Laundry detergent Method was impaired in both PCR and REP-PCR. Efficacy of The boiling and chloroform extraction methods was disrupted only in REP-PCR. No deficiency was observed in Sinagen kit method.

Conclusion: The findings of this study show that despite the availability and lower cost of manual methods, these methods cannot be substitute for kit method.

Keywords: PCR, REP-PCR, Manual Methods, kit, Bacterial DNA extraction

P976: **Effect of initial concentration of ferrous ions in biooxidation of mouteh sulfide gold ore**

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Background and Aim: The term biomining have been coined to refer to the use of microorganisms in mining processes.On the other hand, biooxidation implies the bacterial oxidation of reduced sulfur species accompanying the metal of interest, as in the biooxidation of refractory gold minerals.The main advantages of biooxidation of refractory gold ores as compared with pyrometallurgylie in its relative simplicity, low capital costs, low energy input, and in its friendliness towards the environment. Chemolithoautotrophs are major organisms in biomining process that are able to use ferrous iron or reduced inorganic sulfur sources (or both) as electron donors.

Methods: Different samples, from Chakhaton and Sanjedeh gold mines, Muteh, Iran, were collected and inoculated in the9K liquid medium. Purification and enrichment of acidophiliciron and sulfur oxidizing bacteriawas performedonthe 9k agar solidmedium. The leaching experiments were performed in agitation flasks for 7 days, in which the initial 1% pulp concentration of 150µ ore particle size and bacterial inoculation was 10% V/V. Three Media with different concentration of Fe2+ (9K, 0.9K and 0K media) were used to measure the effect of initial concentration of Fe2+on bioxidation rate. The pH value was adjusted with sulfuric acid to 2 before the inoculation was processed. The temperature and rate of shakewas 30°C and 180rpm respectively. During the leaching, Redox potential and pH were measured daily and ferrous iron concentration was determined by spectrophotometer using 1, 10-orthophenanthroline ferrous complex as an indicator.

Results: The isolated strain (F.O.C.B.)was iron and sulfur oxidizer acidophilic bacteria. This strain was rod shaped and obligate autotrophic. The results showed that isolated bacterium reduced the amountofferrousion from 0.63to 0.015gr/l. The greatest increase of Eh and decrease of pH carried out in 0.9k, 0k and 9k media respectively.

Conclusion: This result showed the effect of initial concentration ofFe2+on bioxidation. Best medium for biooxidation in this study was 0.9K medium.

Keywords: Biooxidation, Isolation, Muteh, Gold,Chemolithotroph

P977: **The effect of monolaurin in combination with Menthe pulegium L. and Menthe spicata L. essential oils on Bacillus cereus and E. coli O157: H7 (in vitro study)**

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Background and Aim: Monolaurin, pennyroyal (Mentha pulegium L., labiatae) and spearmint (Mentha spicata L.) essential oils have various antibacterial activities on microorganisms.

Methods: Enhancement of antibacterial activity and detection of their combined effects on B. cereus ATCC 11778 and E. coli O157: H7 were the purpose of this investigation. Monolaurin preparation, spearmint and pennyroyal essential oils preparation and analysis of their chemical composition with GC-MS method, bacterial inoculums preparation, antimicrobial susceptibility testing using broth micro dilution MIC testing and finally statistical analysis of results with SPSS 17 software package were the material and methods used in this research.

Results: MIC (minimum inhibitory concentration) of pennyroyal oil, spearmint oil, monolaurin, monolaurin-pennyroyal oil combination and monolaurin-spearmint oil combination on B. cereus and E. coli O157: H7 were significant ($p<0.01$). The MIC of monolaurin-pennyroyal oil combination and monolaurin-spearmint oil combination on B. cereus in comparison with the MIC of pennyroyal and spearmint oils separately were significant ($p<0.01$). Monolaurin spearmint oil combination showed synergistic inhibitory effect on E. coli O157: H7. The most effective antimicrobial agents on B. cereus were monolaurin, and monolaurin-spearmint oil combination and monolaurin-pennyroyal oil combination, and the lest effective agent was spearmint oil. Also the most effective antimicrobial agent on E. coli O157: H7 was pennyroyal oil, and the least effective agent was monolaurin.

Conclusion: Since the MIC of monolaurin-pennyroyal oil combination and monolaurin-spearmint oil combination on E. coli O157: H7 in comparison with the MIC of monolaurin separately were not significant, therefore for significant effect on E. coli O157: H7 and other gram negative bacteria, combination of monolaurin with chelating agents and other natural antimicrobials are suggested.

Keywords: monolaurin, menthe pulegium essential oil, menthe spicata essential oil, E. coli O157: H7, B. cereus

P978: **The effect of temperature and pH on catalase production from bacterium Kocuria ASB107**

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Background and Aim: In this study we examined the effect of temperature and pH as a stress on the induction of defense system of radio-tolerant bacterium KocuriaASB107. Nowadays catalase has numerous applications in various industries such as food industry, dairy production, wastewater treatment, and also medical industry; hence it is obvious that purification of catalase from suitable microbial sources is a valuable method in supplying the demands of the mentioned industries. Based on previous reports, Kocuria ASB107, which was isolated from Ab-e-siah spring in Ramsar, has shown a relatively high resistance to ionizing and UV radiation. It is suggested that its extreme resistance to ionizing radiation is attributable to an effective DNA repair system and a special chromosome structure. However, protective mechanisms against oxidative damage may also be involved in this high radiation resistance. The most lethal effects of ionizing radiation is known to be induced by generating hydrogen peroxide and oxygen free radicals which damage the cell components such as DNA and proteins. One of the defense mechanisms of this bacterium is production of large amounts of catalase enzyme which makes it a great candidate for production of the catalase needed in the aforementioned industries.

Methods: The effect of temperature on the catalase activity was examined after incubation of the cells at various temperatures (15, 20, 25, 30, 35, 40, 45, 50, 55, 60, and 65˚C) at pH 7 for 41 hours. Biomass of bacteria was collected in a stage of growth phase where the maximum amount of catalase is produced. After a freeze and thaw period, the biomass was treated with lysozyme enzyme. Cell extract was used for the examination of catalase In order to study the effect of different pHs on catalytic activity, after washing, freezing, and lysozyme treatment of the biomass, cell extract was examined.

Results: The catalase activity increased at 30 and 35?C but the maximum activity was observed at 30?C. Enzyme has a broad catalytic pH range. Kocuria ASB107 displays the highest catalytic activity at pH of 7 to 10.

Conclusion: under the conditions mentioned above, the defense mechanism of this bacterium is the most efficient. Our finding indicates that, this catalase has a high potential for industrial application to remove residual hydrogen peroxide –due to its toxicity for environment and human health-.

Keywords: temperature, pH, catalase, Kocuria ASB107

P979: **Rapid detection of coliform in drinking water by Multiplex PCR in comparison with MPN method**

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Background and Aim: Detection of bacterial contamination in drinking water by culture method is a time and cost consuming method and spends a few days depending on contamination degree. However the people serve the tap water at that time. Molecular methods are rapid and sensitive. In this study a rapid Multiplex PCR method was used for rapid analysis both coliform bacteria and E.coli ,and probable detection of VBNC bacteria in drinking water, the experiments were performed in bacteriological lab of water and Wastewater Corporation in Markazi province.

Methods: Rapid detection of coliform bacteria was examined by amplification of lacZ and uidA genes in a Multiplex PCR. 80 samples was taken from Arak drinking water system including 41 samples of wells, 36 samples of water distribution network and 3 samples from water storages were examined by amplification of lacZ and uidA genes in a Multiplex PCR. Equivalently, the MPN test was applied as a standard method for all samples for comparison of results. Standard bacteria, pure bacteria that isolated from positive MPN and CRM were examined by PCR and MPN method.

Results: The result of most samples water network, water storages, and water well were same in both MPN and PCR method .The results of standard bacteria and pure cultures of bacteria isolated from positive MPN and CRM confirmed the PCR method. Five samples were positive in PCR but negative in MPN method. Duration time of PCR was decreased about 105 min by changing the PCR program and electrophoreses factors.

Conclusion: The Multiplex PCR can detect coliform bacteria and E.coli synchronous in drinking water.

Keywords: Comparison, PCR method, Bacterial contamination, Drinking water, MPN method

P980: **Ethanol-producing bacteria from natural habitats: Isolation, identification and activity**

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Background and Aim: Screening for ethanol-producing bacteria from natural resources and assay of their ethanol production to produce higher ethanol as a replacement for fossil fuels has an ignorable importance in the world future energy trends. In this research, natural environments samples such as fruits, sap of plants, soils and the alcohol industries were collected to isolate ethanol producing bacteria.

Methods: First, different samples were cultured in RM broth then transferred on RM solid medium. Different environmental factors such as agitation (oxygen), nitrogen and carbon Source, temperature and pH were investigated.

Results: 9 out of 81 isolates that produce high ethanol were selected for further studies. Ethanol producing strains are rod shape (Zym1-Zym8) and circle shape (Zym9). Results showed that ethanol producing bacteria used xylose and tryptophan as best sole sources of carbon and nitrogen, respectively. Furthermore the optimal pH and temperature were assayed 6 and 35°C, respectively. High ethanol-producing isolates was Zym2, Zym7 and Zym8 with more than 10%.

Conclusion: These bacteria were able to produce high yield of ethanol and could introduce to ethanol production industries.

Keywords: Ethanol, Bactria, Fermentation

P981: **Biodiversity and 16S Ribosomal RNA application in space biotechnology**

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Background and Aim: Modern space biotechnology is a wide term which can cover a wide range of ways of changing the genetic material - the DNA code - in a living organism.

Methods: Preparing suit Microorganism needs complete information of earth microorganism's population. The comparison of rRNA sequences is a powerful tool for deducing phylogenetic and evolutionary relationships among bacteria, archaebacteria, and eucaryotic organisms.

Results: A set of oligonucleotide primers capable of initiating enzymatic amplification (PCR) on a phylogenetically and taxonomically wide range of bacteria is described along with methods for their use and examples.

Conclusion: We name 16S Ribosomal RNA as The molecule" of space biotechnology

Keywords: 16S rRNA – diversity -ARDRA – Archia –Halophytes

P982: **Induction of expression of dszABC genes from Gordonia sp. in E.coli**

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Background and Aim: Crude oil contains numerous sulfur compounds in different forms. Combustion of fossil flue result in the release of sulfur dioxide (SO2) in to the environment that causes air pollution and acid rain. There are two methods for desulfurization including Hydrodesulfurzation(HDS) which is carried out under high temperature and high pressure and biodesulfurization(BDS) that is done by microorganisms. BDS is less expensive in comparison with HSD and can be used under normal condition.4S biochemical pathway in microorganisms has the most important role in biodesulfurization and enzymes of this pathway are responsible for reactions which are happened in BDS. Biodesulfurization enzymes are coded by dsz operon. This operon contains 3 genes dszA, dszB and dszC. Gordonia sp. that has capability to desulfurize oil was isolated from oil polluted soil of Khozestan. In this study, we investigated ability of expression of dszABC genes in E.coli from Gordonia sp. for using in oil industry.

Methods: First of all, specific primers based on sequences of dsz operon in gene bank were designed. Then, the dsz ABC operon was isolated using these primers and was cloned in pET43.1a+ expression vector. After that, it was verified by sequencing. pET43.1a+- dszABC vector was transformed in to E.coli BL21. For expression of these genes, the recombinant strain was cultured in TB broth supplemented with 50µg/ml ampicillin. E.coli which contains vector without genes was used for negative control. The induction was done by IPTG (1mM final concentration) as inducer. Samples were taken before IPTG induction and at 1, 2, 4, and 8 h after the induction. The result of expression was analyzed by 12.5% SDS-PAGE and DOT blot.

Results: The SDS-PAGE analysis showed a protein band in induced cells in comparison with non-induced cells and it was in the range of 30-35kDa of the protein size marker which it was close to the size of DszB. The DOT blot confirmed the expression of dszC.

Conclusion: Because of being His tag and HSV tag in the vector and position of dszC in the operon the expression of 2 other genes also can be confirmed by Dot blot. Furthermore, the SDS-PAGE shows the protein bond of DszB which confirms the expression of dszA (because of position of dszB in operon). The expression of this operon in recombinant strain shows E.coli is a suitable microorganism for optimizing biodesulfurization process in future.

Keywords: Biodesulfurization, dsz operon, Gordonia sp., Gene expression, SDS-PAGE , DOT blot

P983: **Determination of lactic acid bacteria and yeasts in local yogurts of Gilan province in 2012**

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Background and Aim: Lactic acid bacteria and yeasts as starter organisms is responsible for production of yogurts from milk. Majority of probiotic groups are belong to this type of bacteria that by improvement of beneficial microorganisms of human digestive tract, cause the human immune system boost and diseases prevention such as cancers, infections, asthma and skin allergies. Scientists believe that the number and diversity of yeasts and lactobacilli is very effective on the quality of the local yogurts of villages. In this study, local yogurts of different villages in Gilan province in terms of Lactic acid bacteria and yeast were studied, because isolation and identification of beneficial microorganisms for use in dairy industry of Iran will result independency of this industry for importing of starter strains from other countries.

Methods: In this study, from 10 villages of Gilan province were randomly 150 samples selected. Sampling in the laboratory was done by sterile spoon from middle part of yoghurt container and then samples were diluted by the ratio of 1 in 10 and up to 108 were diluted. 100 µl from 10-7 and 10-8 dilutions of each samples were added to MRS agar medium and were incubated at 37◦C in anaerobic conditions. Also 100 µl from 10-4 and 10-5 dilutions were taken for yeast culturing in SDA medium at room temperature conditions. After colony growth, the number of microorganisms per ml were determined. After purification of Lactobacilli, species were identified by Gram stain, catalase test and sugar fermentation methods.

Results: The obtained results from different local yogurts of Gilan's villages showed highly numerous different types of microorganisms that the number of yeasts in per liter of yoghourts was 56×103 cfu/ml and the number of lactobacilli was 60×107cfu/ml. Among these microorganisms, Lactobacillus plantarum and Lactobacillus bulgaricuss were most frequent lactobacilli and Saccharomyces cerevisiae and then Saccharomyces Boulardii had the maximum number of yeast.

Conclusion: Based on the obtained results in the local yogurts of Gilan villages, there are numerous amount of probiotic microorganisms, which is the main reason for people's health of this area. It is appropriate to inform the people of rural and urban areas of the province and spread out the production of these local yogurts to increase of population health factors of these areas. In this study number of probiotic microorganisms in local yogurts of villages had significant differences. We propose a further research on local yogurts of villages that has largest probiotic microorganisms to isolate the best strains as starter of dairy companies and it may leads to independency of dairy industry from imported products.

Keywords: Gilan province, Local yogurt, yeast, Lactic acid bacteria

P984: **Optimizing of the conditions for organic solvent extraction of persimycin by response surface methodology**

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Background and Aim: Investigation of the Streptomyces sp. UTMC 1154 metabolites led to isolation of new cyclic peptides named persimycin A and B. An optimized condition of the antibiotic extraction guarantees reproducible and trustful results and reduces the extraction time, efforts and expenses. These reasons trigger a motivation for developing an optimized extraction method to isolate persimycins for further works.

Methods: Fermentation medium of Streptomyces sp. UTMC 1154 contained soybean 30 g/L, starch 20 g/L, CaCO3 15 g/L, and MgSO4.7 H2O 2 g/L at pH 7-7.2 and was harvested after 7 days. The whole broth was extracted by liquid solvent extraction using butanol. Effects of several factors and their interactions, including ratio of organic solvent to fermentation broth, process temperature, extraction time, shaking rate and solution pH were investigated using half fractional factorial and central composite designs. Chromatographic separation was performed on C18, flow rate of 0.8 mL/min and UV detection at 210 nm. Deionized water and acetonitrile were used as mobile phase.

Results: Extraction time and shaking rate were found as significant factors while ratio of organic solvent to harvested fermentation medium had significant interaction with shaking rate. By solving the quadratic regression model equation using ANOVA, the optima of the variables were determined as: extraction time, 59 min, shaking rate, 250 rpm, and organic solvent to fermentation ratio, 1.1: 0.9 v/v. The peak area was elevated from 1375 to 1743 which showed 27% increase in extraction efficiently.

Conclusion: The extraction of novel antimicrobial cyclo-pentapeptide, persipeptide, produced by Streptomyces sp. UTMC 1154 was found to be extremely influenced by several physico-chemical parameters. It is suggested to use proposed optimized condition reported here for optimization studies of the production media of persimycins.

Keywords: Persimycin, HPLC, Extraction, Central Composite Design, Response Surface Methodology, Half Fractional Factorial Design.

P985: **Identification and Characterization of an Escherchia coli Strain QW184 That Reduces Selenate to Elemental Red Selenium**

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Background and Aim: Selenium is an essential element for humans and animals in trace amounts but it is toxic at higher levels. The accumulation of selenium in certain environments requires the development of efficient detoxification processes. Selenite (SeO32-) and selenate (SeO42-) are the two soluble species of selenium that can be found mostly in aerobic habitats. Various species of bacteria reduce selenate to elemental selenium (insoluble and nontoxic). Thus, bioremediation has been considered as an effective means of cleaning up of selenium-contaminated sites. Also, Heavy metals-resistant often relate to antibiotic-resistant.

Methods: In this study, 30 strains were isolated from wastewater samples collected from selenium-contaminated sites in Iran using the enrichment culture technique and direct plating on agar. The disk diffusion method was performed to determine resistance or sensitivity for this bacterium, and that was identified as Escherchia coli (AJ567606) by conventional biochemical tests and 16S rRNA gene sequencing.

Results: Escherchia coli strain QW184 exhibited very high MIC values for selenate (700 mM). The strain reduces toxic selenate anions into red elemental selenium under aerobic conditions. Also, bacterial strain was resistant to Penicillin, polymyxin B, Gentamycin, Vancomycin and Erythromycin and exhibited Intermediate to Cefazolin and streptomycin.

Conclusion: The genetically modified bacteria carry a plasmid containing genes conferring resistance to the antibiotics as well as resistance to toxic oxyanions of selenium. These microorganisms could be further used for bioremediation of contaminated sites.

Keywords: Escherchia coli, MICs, Selenate, resistant, bioremediation

P986: **Comparison of pure and mixed cultures of chemolithotroph iron and sulfur oxidizers rods and spirals shape bacteria in biooxidation of mouteh sulfhide gold ore**

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Background and Aim: Bioleaching is considered to be anefficient and an ecological friendly process commonly used by the miners as an alternative method to roastingor smelting, especially about low grade ores. In this process the bacteria act as a catalyst to accelerate the natural processes inside the ore. The bacteria use a chemical reaction known as “oxidation reaction” to convert ferrous iron into ferric iron and sheer metals. Most frequently isolated within mining environments belong to the genera of Acidithiobacillus and Leptospirillum. Acidithiobacillusis rod shape and Leptospirillumis aspiral form of chemolithotrophic ferrous oxidizing bacteria. Bioleaching of sulphide minerals is an established technique for the recovery of gold from their sulphide ores. The mineral used in this study was Muteh sulfide gold ore. The main mineral composition of this ore was pyrite and arsenopyrite, therefore, removalof these minerals through biological oxidation does it feasible for extracting using cyanide.

Methods: A total of 10 samples collected from Chahkhatoon and Senjedeh minerals and dumpsin muteh mining area, Isfahan, Iran. 10gr of each sample inoculated in selective media and incubate under aerobic conditions at 30°C for 7-21days. The 9K agar used for isolation and morphological studies. Ferrous iron was determined by spectrophotometer using 1, 10Orthophenanthroline. The bioleaching experiments were carried out in 250ml Erlenmeyer flasks. The flasks were filled with 90 ml of 9K and DSMZ882 media and 1% (w/v) of ores and inoculated with 10% of pure or mixed culture of isolated bacteria. The initial pH of the solution was adjusted to 2.0 with H2SO4.In order to evaluatethe behavior of mixed culture of bacterial species on bioleaching reactions,mixed and pure cultures of isolated bacteriawere prepared. All experiments were done and carried out in rotatory shaker at 180rpm, 30°C for7 days. Eh, pH and ferrous iron concentration were measured daily.

Results: The isolated bacteria in this study, were included a variety of oxidizing acidophilicautotrophic iron and sulfur oxidizing that named F.O.C.B and C.L.L.B. After 7 days of incubation, ferrous iron concentration in culture of F.O.C.B reduced from 0.64to 0.05g/l and in culture of C.L.L.B reduced from 0.67to 0.04g/l. Changes of ferrous iron concentration in mixed culture was from 0.66 to 0.006 g/l also the result of Eh and pHin mixed culture was better than individual strains.

Conclusion: Results showed that mixed culture of iron oxidizing bacteria were useful for bioleaching process.

Keywords: bioleaching. mouteh.refractory gold ore.biooxidation

P987: **Enhancement of recombinant human α-synuclein production by using propionic acid as acetate competitive inhibitor**

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Background and Aim: Escherichia coli produce acetate throughout exponential growth in glucose supplemented medium and it is the most important obstruction to production of recombinant proteins. Numerous approaches have been challenged for reducing acetate production including modification of the medium conditions and carbon source as well as employing the engineered strains. The aim of this study was to investigate the propionic acid effect on the redistribution of acetate pathway and the production yield of human α-synuclein recombinant protein.

Methods: The accumulation of acetate, pH values, lactate concentrations, bacterial growth and the yield of human α-synuclein recombinant protein were monitored when low concentrations (20-200 mM) of propionic acid were added to the culture medium which was supplemented by glucose.

Results: Results of HPLC indicated that the amount of acetate in treated medium by propionic acid was much lower than control which demonstrated such supplement might be able to redirect glucose flux from acetate pathways to less inhibitory byproducts because it is known as an inhibitor of bacterial acetate kinase. Assessments of lactate also proved redirection of the carbon flux toward less inhibitory by-products. In addition results indicated that there was a positive relation between the pH of the culture and the acetate accumulation during growth. pH was more stable in the supplemented sample. Furthermore, significant results were obtained for expression of ?-synuclein and bacterial growth which confirms removing toxic effect of acetate by using such supplementation.

Conclusion: Propionic acid can be a useful candidate as a supplementation in culture medium for redirection of metabolic pathway in order to enhancing recombinant protein production with no need of complex genetic manipulations.

Keywords: Acetate, Alpha synuclein, Propionic acid, Recombinant protein

P988: **Biosorption of Cement factory lead using Saccharomyces carlsbergensis biomass**

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Background and Aim: The heavy metal pollution has become one of the most important environmental problems. Heavy metals are the most important environmental pollutants, especially for human health and aquatic systems are a serious threat

Methods: Iranian Research Organization for Science and Technology of yeast Saccharomyces carlsbergensisPTCC 5051, and received as a lyophilized culture YEDPA environment and then the proliferation of malt extract broth is used. The effects of pH, temperature, kinetics and isotherm of lead on metal uptake were evaluated by the SC. Absorption maximum at about pH 5, the optimum temperature was 25 degrees Celsius.

Results: A kinetic study showed that batch biosorption of lead to rapid removal by the biomass of Saccharomyces was the first test was done in less than 30 minutes. Using FT-IR Method, surface functional groups of fungi were identified. With active and passive absorption of lead by Saccharomyce, It has revealed that most absorption takes place by active yeast .With yeast and control yeast on the autoclave, the pre-treated with sodium azide and 2, 4 Dinitrophenol (DNP)showed that claims of capturing, respectively, 0.68, 0.12, 0.44 and 0.48 Mmol gram was obtained.

Conclusion: Results showed that, even using the passive yeast are suitable for the Bioabsorption of lead.

Keywords: Bioabsorption, lead, Effluent, Saccharomyces carlsbergensis

P989: **Genetic Engineering of Microalgae for Enhanced Biodiesel production**

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Background and Aim: Due to negative environmental influence and limited availability, petroleum derived fuels need to be replaced by renewable biofuels. Biodiesel has attracted intensive attention as an important biofuel. Microalgae provide various potential advantages for biodiesel production when compared with 'traditional' crops. Specifically, large scale microalgal culture need not compete for arable land, while in theory their productivity is greater. In consequence, there has been resurgence in interest and a proliferation of algae fuel projects.

Methods: There are a series of consecutive processes for biodiesel production with microalgae as feedstock, including selection of adequate microalgal strains, mass culture, cell harvesting, oil extraction and transesterification. To reduce the overall production cost, technology development and process optimization are necessary.

Results: Genetic engineering also plays an important role in manipulating lipid biosynthesis in microalgae. Many approaches, such as sequestering carbon dioxide from industrial plants for the carbon source, using wastewater for the nutrient supply, and maximizing the values of by products, have shown a potential for cost reduction.

Conclusion: However, while on a theoretical basis, microalgae may produce between 10 and 100 fold more oil per acre, such capacities have not been validated on a commercial scale. This review provides a brief overview of genetic engineering of microalgae for enhanced biodiesel production.

Keywords: biofuel, biodiesel, microalgae, Genetic engineering.

P990: **Optimization of cholesterol oxidase expression in E. coli by Response Surface Methodology**

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Background and Aim: Cholesterol oxidase (CHO) is a flavoprotein that has a great commercial value, widely employed by laboratories, routinly devoted to the determination of cholesterol concentration in serum, other clinical samples and foods. CHO is a biofunctional enzyme that catalyzes the oxidation of cholesterol to temporary intermediates and produce hydrogen peroxide. CHO has two forms, and both of them have no significant homology. To reduse time and cost of optimization, response surface methodology (RSM) was employed for optimization of a recombinant CHO from Rhodococcus equi PTCC 1633.

Methods: In this work, we investigate optimal condition of cholesterol oxidase expression in E. coli. We use three-level four-parameters including, induction time, IPTG concentration (amount of inducer addition), time of incubation and temperature, by Box-Behnken Design using minitab 15 software for optimization.

Results: A recombinant cholesterol oxidase has been over-expressed in BL21(DE3) E. coli cells. Early results demonstrated that maximum production of cholesterol oxidase was achieved at room temperature and high level of IPTG concentration (amount of inducer addition. Optimization of expression condition including induction time, IPTG concentration, time of incubation and temperature, represent the factors concurring to achieve the optimal expression level. Notably, this expression level is higher than previously described production of cholesterol oxidase.

Conclusion: The optimal condition of the expression of cholesterol oxidase for maximum cholesterol oxidase yield was determined using statistical experimental design and analysis. By altering expression condition at flask level, an improved microbial process was set up giving several fold improvement in expression.

Keywords: cholesterol oxidase, Response Surface Methodology, optimization, Rhodococcus equi

P991: **Microbiological control of cosmetics products during 3 years since 1389**

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Background and Aim: Due to high rate of worldwide using cosmetic products especially in Iran, and the importance role of their microbiologic contamination in adult and children health,standard microbiology control of these products is the principal duties of the ministry of health and education.

Methods: Specification and test method Based on Microbiology methods of national standards of Iran No.11068,11169,9793,11804,3978,3271,6342,9934 and 9933 performed on 1400 cosmetic specimens which received during 3 years.Total aerobic and mesophilic bacterial count, Mold and yeast count, E.coli, Pseudomonas and Staphylococcus aureus detection performed for all specimens.

Results: 2.1 percent of specimens were rejected. The rejected samples included mascara, hand cream and fruit conditioners.

Conclusion: Low percent rejection rate shows that the specimen release to market from legal way,may has high quality, and the producer and importer knows the law and requirements, so present better products compare with illegally market products.

Keywords: Cosmetic products, Microbiology specification, Microbiology control

P992: **Microbiological control of Minced meat, Hamburger, Chicken nugget, Meat paste**

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Background and Aim: Processed and ready to use foods use more and more every day in the entire world with many kind of consumer. This type of foods need short time to heat before consumption. Usually they are contaminated with some pathogen bacteria during not suitable preparation. Due to this fact and more probability to microbial contamination, this study was accomplished to control microbial quality of this type of food.

Methods: this study was fulfilled based on the national standard (NO. 2946-1810-6806-6805) for detection of E.coli, E.coli O157: H7 , Salmonella and Staphylococcus aureus in 30 samples .

Results: Findings of the survey showed 29 (96.66%) of the total samples were contaminated with at least one of studied bacteria and weren't appropriate to use , 28(93.33%) have E.coli, 1(3.3%) have Salmonella, 14(46.66%) have S.aureus and 2(6.6%) have E.coli O157: H7.

Conclusion: Results indicated that the method of production of this type of foods have not good quality , and since various reports claim of inappropriate health quality of these products , more supervisions and more serious of health authorities are required to production to improve the safety of respect foods.

Keywords: microbiological quality , nuggets , Hamburger

P993: **A comparison of type and rate of optimal nitrogen source to produce Lactic acid by L.plantarum bacteria and R.oryzae fungus**

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Background and Aim: Lactic acid is produced for use in various industries has always been considered by researcher. This acid is an important commercial acid as the primary metabolite that has high application in food, pharmaceutical and chemical industry. Lactobacillus plantarum PTCC: 1058 bacteria and Rhizopus oryzae PTCC: 5263fungus have highest rate of acid production between microorganisms of its group. In this study, the best and most suitable nitrogen source to produce the highest concentration of lactic acid in the culture medium, was studied for the two microorganisms and compared to each other.

Methods: In this study, two series of medium, submerged and solid state, to culture R.oryzae fungus were used. Submerged medium was potato dextrose agar and solid state medium was prepared by bran. The strains were inoculated into the medium and incubated for 48 h, at 200rpm and 30°C. L. plantarum bacteria are cultured on solid slant MRS medium, then grown Lactobacillus cells transferred to pre-cultured medium(Liquid MRS) and ultimately, for acid production, the cells are transferred to final culture medium. Culture medium with different nitrogen sources such as KNO3, NH4Cl, Corn steep, NH4NO3, NaNO3, yeast extract, peptone, for both microorganisms were prepared and acid production was investigated. for estimating of lactic acid, colorimetric assay method was used.

Results: Results revealed that the highest amount of acid in R.oryzae fungus were produced in culture medium with NH4H2PO4 nitrogen source. We would have the highest amount of acid with 3gr/l of NH4H2PO4 in the medium, and maximum amount of acid was equal to 107g/l. Corn steep with 15g/l concentration in culture medium was optimal nitrogen source for L.plantarum bacteria that produced maximum amount of lactic acid equal to 59g/l.

Conclusion: Using of fungus strain of R.oryzae and nitrogen supplementary of NH4H2PO4 leading to production of maximum amount of acid107gr/l by 89% yields, which this amount is consequently higher than the amount of acid produced by L . plantarum bacteria. Moreover NH4H2PO4 is a simple chemical compound and a key advantage over corn steep, because it does not have complex purification such as corn steep that requires complicated steps to remove impurities and therefore it makes higher production and purity for us. It seems that R.oryzae 5263 is a suitable case for research activity by aspect of commercial production of lactic acid that is highly applicable in various industries, including food industries.

Keywords: Rhizopus oryzae, Lactobacillus plantarum, Nitrogen source, Corn steep, NH4H2PO4, Lactic acid.

P994: **Isolation and identification of Acidithiobacillus ferrooxidans ATCC23270 from uranium mine of Gachin port in Bandar Abbas, Iran**

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Background and Aim: Microbial oxidation and reduction of iron and sulfur are important parts of bioleaching process. Species of Acidithiobacillus ferrooxidans is the common ferrous-iron and sulfur oxidizers. This ability makes it suitable for use in biomining to recover metals such as gold, copper and uranium. In the present study, Acidithiobacillus ferrooxidans ATCC23270 was isolated from uranium mine of Gachin port in Bandar Abbas and identified by 16SrDNA sequences.

Methods: The water samples were collected from uranium mine of Gachin port in Bandar Abbas, Iran. The strains were cultivated in 250-mL flasks containing 100 mL of 9K medium in a shaker incubator at 180 rpm at 30 0C. The initial pH of the medium was adjusted to 2.0. After bacterial growth, purification was done as follows: sample was inoculated on to individual 9K plates and incubated at 30 0C for 4–6 days until the colonies appeared. The transfer was repeated until the pure culture was obtained. A single colony was then picked out for next inoculation. Their identities were confirmed based on their phenotypic characteristics and 16SrDNA sequences.

Results: The bacteria in the fresh sample were grown after 60 day incubation. Following three transfers on the 9K plates, we obtained pure cultures of strain after 5 days. The colonies were brick red and fine. This isolated were Gram-negative, motile, acidophilic and chemolithoautotrophic bacterial rods and could gain energy by the oxidation of Fe2+, S0 and pyrite. On the basis of 16SrDNA nucleotide sequences similarity, the bacteria showed sequence similarity of 99.9% to ATCC23270.

Conclusion: Acidthiobacillus ferrooxidans strain ATCC23270 was isolated from uranium mine of Gachin port in Bandar Abbas, Iran. It was gram negative and could use ferrous and sulfur as the sole energy source . Optimum pH and temperature for growth were 2.0 and 30 ℃, respectively.

Keywords: Acidthiobacillus ferrooxidans; Bioleaching; Isolation; Identification

P995: **The comparence of 4 industrial Ethanol producing Saccharomyces cervisiae strain in synthetic and industrial media**

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2- shahid chamran university

Background and Aim: Saccharomyces cervisiae can have four fermentation pathways in different conditions, and the most important one is Alcoholic fermentation. For several industrial applications and the reservation of potential energy of glucose in ethanol product, this metabolite is one of the valuable materials for different industries and even is considered as better fuel. Other way molasses is an adequate source for producing Ethanol, because it has large amount of sugar in Iran. In laboratory surveys of ethanol fermentation, those culture media which their N & P sources are yeast extract and pure material salt are usually used. But is it possible to apply the results of these laboratory surveys in industry, and will the strain in laboratory synthetic media which have highest productivity, function similarly in industrial media?

Methods: To measure this thesis, 4 industrial strains compared in synthetic and industrial media. The synthetic media are consistent of cane molasses 15 brix (sugar concentration: 95 g / l), yeast extract 5 g/l, ammonium chloride 1.5 g/l, magnesium sulphate 4 g/l, potassium chloride 1.7 g/l and potassium dihydrogen phosphate 5 g/l. For preparing the industrial media, at first the sugarcane molasses of Khuzestan and the industrial sources of Nitrogen & Phosphate were analyzed. Then, based on the components of molasses and purity percent of the source of Nitrogen & Phosphate and also the general formulation of yeast, the component of culture media according to the productive industries was calculated. (The C & N and P sources were selected according to industrial plant sources exactly). The mentioned cultures media are consistent of cane molasses 15 brix (sugar concentration: 95 g / l), industrial urea(2 g/l), industrial ammonium sulphate(2 g/l) and diammonium phosphate(3 g/l). Then inoculumed 2% v/v of preculture media. Sampling of the culture media was done in 4 hours intervals and under the sterile condition. The production of ethanol and the consumption of sugar were measured. Ultimately the quantity of the yield, efficiency and productivity were computed and analyzed in ANOVA analysis.

Results: The results of this study showed that for all studied 4 strains in industrial media compared with synthetic media, yield, efficiency and productivity quantity decreased, that indicates the importance of N & P source difference in these media. It is worth mentioning that strain 4, has higher yield, efficiency and productivity in the synthetic media in compared to strain 3, however, strain 3 preceded the strain 4 in industrial media, which indicates in adaptive ability of the strains in relevance to industrial media.

Conclusion: Therefore, by investigating for one strain in synthetic media, the amount productivity can not be found in industry, and it is more effective to use identical media with industrial media to screen industrial strain and investigate their adaptive ability.

Keywords: Alcoholic fermentation, Saccharomyces cerevisiae, sugarcane molasses, adaptive

P996: **Evaluation of bacterial contamination (Lactobacillus plantarum PTCC 1058) effects on industrial process of Ethanol production in Lab scale**

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Background and Aim: Nowadays, there is a bioethanol which is still considered as one of the most important biotechnology product. Moreover, fermentation, which is basic to these processes, will lead to the generation of beneficial products because of the microorganism activities especially yeasts. The Saccharomyces cerevisiae yeast is the most applicable one in this industry. Besides, molasses is an adequate source for producing Ethanol, because not only is cheap and abundant, but also it has large amount of sugar in Iran. The purpose of study is the effect of bacterial contamination with Lactobacillus plantarum PTCC 1058 for ethanol production in industrial process.

Methods: At the first the sugarcane molasses of Khuzestan and the industrial sources of Nitrogen & Phosphate were analyzed. Then, based on the components of molasses and purity percent of the source of Nitrogen & Phosphate and also the general formulation of yeast, the component of culture media according to the productive industries was calculated. (The C & N and P sources were selected according to industrial plant sources exactly). The mentioned cultures media are consistent of industrial urea(2 g/l), industrial ammonium sulphate(2 g/l) and diammonium phosphate(3 g/l). Considering previous optimization results, this study was performed with cane molasses 17.5 brix (sugar concentration: 110 g / l) and inoculum's size 4%. for studying the effect of bacterial contamination, the Mc farland tubes as a scale for measuring concentration of the intended bacteria were utilized and inoculumed with different concentration (3×108, 6×108, 12×108 and 24×108) from Lactobacillus plantarum PTCC 1058. A blank and uncontaminated sample was compared with other samples. sampling of the culture media was done in 4 hours intervals and under the sterile condition. The production of ethanol and the consumption of sugar were measured. Ultimately the quantity of the yield, efficiency and productivity were computed and analyzed in ANOVA analysis.

Results: The results of this study showed that the presence of the studied Lactobacillus plantarum in concentrations stronger than 6×108cfu/ml cause a reduction in the produced ethanol and fermentation productivity.

Conclusion: The concentration 6×108cfu/ml from Lactobacillus plantarum PTCC 1058 is effective concentration threshold.

Keywords: Alcoholic fermentation, Saccharomyces cerevisiae, sugarcane molasses, Productivity, Lactobacillus

P997: **Effect of cross-linking condition on immobilization of whole-cell biocatalyst**

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Background and Aim: It is a long time that biocatalysis are of increasing importance for using in chemical and pharmaceutical industries. Microbial cells are the best candidates for using as whole-cell biocatalyst especially in immobilized form. Cross-linkers (such as glutaraldehyde) can help to form cross-linkage between cells and suitable supports via binding of chemically functional groups in the surface of the cells and the supports. In the present study, the effect of various concentrations of glutaraldehyde and different incubation times was investigated on efficiency of bacterial immobilization on egg-shell surface as support.

Methods: Kocuria ASB 107 bacterial cells with high catalase activity were used as biocatalyst. Egg shell was mixed with potassium phosphate (50 mM, pH 7.0) contain of wet cell paste. Different concentrations of glutaraldehyde were added to the mixture slowly. Then the immobilization was checked by measuring catalase activity before and after immobilization. Then the best concentration was used for studying the time dependency of immobilization.

Results: Our results showed that both lower and higher concentrations of glutaraldehyde decreased the immobilization efficiency. The optimum concentration of glutaraldehyde was 0.8% (V/V). Moreover, the best incubation time for the cross-linking was determined to be 10 hours.

Conclusion: The most of chemical compounds applied as cross-linker in immobilization, are toxic for bacterial cells. This toxicity is depending on cross-linker concentration and time of incubation. Therefore determination of the optimum values is important. Ten hours and 0.8% are the optimum values for immobilization of Kocuria ASB 107 bacterial cells on egg shell surface.

Keywords: immobilization, cross-linking, whole-cell biocatalyst, bacteria

P998: **Kinetics studies removal of heavy metals from industrial wastewater using bacterial biomass**

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Background and Aim: Heavy metal ions have been known to be widely discharged into environment and water bodies from industries such as metal plating, pigments, batteries, electrical, tannery, paints, oil refining, and chemical manufacturing. Biosorption, the process of passive cation binding by dead or living biomass, using low-cost biomaterials and microorganisms for removing and recovering toxic metals from industrial wastewaters, has emerged as a potential alternative method to conventional techniques. Biosorption as a biotechnology for removal of heavy metals pollution from aqueous solutions has been extensively studied and most biosorption research mainly focused on the process kinetics.

Methods: Microorganisms were isolated from effluent of an industrial zone of Arak, Iran (Kheir Abad Industrial Zone). Bacterial screening was performed from four different location of the zone. To isolate microbes from sewage, bacteria were isolated through serial dilution method on various media. Few colonies of bacteria due to high metal concentrations were observed. The metal resistant bacteria were isolated and amplified. The amplification was performed using biomass production. For this purpose, a medium of agar was used and then, the colonies were washed from the agar surface and were collected. The bigger colonies were identified with microbiological method and then reproduced. Inoculation rate was selected to be 15% of the volume of wastewater in the reactor. The experiments were done in an internal and external loop airlift bioreactors with and without using packed bed. Biosorption of lead, nickel, zinc and cadmium from industrial wastewater by Bacillus, Pseudomonas, Klebsiella and Escherichia coli was studied in an internal and external loop airlift bioreactor with and without using packeh bed. The pseudo-first-order, pseudo-second-order, Elovich and intraparticle diffusion kinetic models were applied to study the kinetics of the biosorption processes.

Results: Results showed that biosorption is a useful method for removal of heavy metals. Also, isolated bacteria used in this study were capable to adsorption of heavy metals. Conformity between experimental data and models predicted values was expressed by correlation coefficients (R2, values close or equal to 1). A relatively high R2 value indicates that model successfully described kinetics of heavy metal biosorption.

Conclusion: Biosorption of lead, zinc, cadmium and nickel in an internal airlift bioreactor with Bacillus, Pseudomonas, Klebsiella, and Escherichia coli in internal and external airlift bioreactor with and without using packed bed was investigated. In order to investigate the mechanism of biosorption, characteristic constants of adsorption rate were determined at different time by using a pseudo-first-order, pseudo-second-order, Elovich and intraparticle diffusion equation. Biosorption of heavy metals onto bacterial biomass in all bioreactors obey the pseudo-second order kinetics, which suggests that the rate-limiting step of this sorption system may be chemical sorption or chemisorptions involving valency forces through sharing or exchange of electrons between sorbent and sorbate.

Keywords: biosorption, kinetic models, airlift bioreactor, heavy metal,Klebsiella,Escherichia coli

P999: **Nickel and Zinc Biosorption and Kinetic Study in a Bubble Column by Bacillus, Pseudomonas, Klebsiella, and Escherichia coli**

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3- Chemical Engineering Department, Arak University, Arak, Iran

Background and Aim: Heavy metals are among major pollutants of water resources. High concentration of these metals has dangerous impact on environment, especially human's life. Industrial waste water is main origin for heavy metals. A variety of methods have been applied to remove heavy metals from industrial effluents. These methods have been effective and they have been able to efficiently remove heavy metals, although much has been argued about the disadvantages of these methods, which can be summarized as costly, not environmentally friendly and usually dependent on the concentration of the waste. Biological methods such as biosorption then were introduced to solve these problems. In early 1980, some microorganisms were observed to be able to adsorb metals . Pseudomonas sp. was used to remove metals such as Chromium, Cadmium, Nickel, and copper. Bacillus also could remove lead in combination with other metals even with a higher rate; while with metals like copper and chromium the result was reverse . Another type of bacteria which was found to be useful in removal processes was Klebsiella. The capsule which this type of bacteria has makes it potentially perfect to adsorb the metals on its surface .

Methods: In this study ,To access efficient microorganism, samples were taken from effluent of an industrial zone of Arak, Iran (Kheir Abad Industrial Zone). Samples then were closely investigated in order to find the indigenous microorganism that could survive in the medium of high-density heavy metals. from four points of a Waste Water Treatment Plant some gram positive and gram negative bacteria under heavy metal stress conditions were isolated by microbiological methods. Biosorption experiments were conducted in bubble column containing waste water in high concentrations of Nickel and Zinc inoculated by isolated bacteria. A kinetic study was done to investigate the fitting of either first-order or second order equations.A 96-percent removal of zinc and a 54-percent removal of nickel were achieved by biosorption column experiment by the isolated bacteria. A comparison between a non-aerated and the aerated column results with the same contact time showed a higher removal percentage in column aerated experiment. The study of contact time in the experiments also confirmed that in a more contact time while the removal efficiency increases the capacity of microorganism to adsorb the metal ions decrease.

Results: Results of kinetic study showed second order with a coefficient of determination of 0.96 and 0.99 for Zinc and Nickel, and the first order with 0.24 and 0.44 for them, respectively.

Conclusion: The results showed that the isolated bacteria could efficiently remove zinc and nickel; therefore, it is recommended for any further biological metal removal process.

Keywords: heavy metals, nickel, zinc, biosorption, kinetic, bubble column

P1000: **Kinetics studies on the biosorption of Nickel and Zinc from industrial wastewater using klebsiella**

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Background and Aim: Introduction: metals, including inorganic contaminants in drinking water are considered dangerous due to the presence of high amounts of waste flows and their stability in the environment, their treatment is essential. Uptake by bacteria (Biosorption) is one of eliminating pollutants.

Methods: In this study, heavy metals nickel and copper from industrial wastewater treatment plants were investigated Arak Kheirabad area. In this study, the four points of the waste water treatment were sampled. Bubble column bioreactor wastewater contains a concentration of 20 ppm of nickel and zinc were inoculated with bacteria isolated. To study the kinetics of the reaction, concentration data with pseudo-first-order and pseudo-second-order equation was evaluated. Findings by bacteria isolated in a bubble column bioreactor removed 96% zinc and 54% nickel, respectively.

Results: Kinetic study showed that the standard deviation charts for biological uptake of nickel pseudo first and pseudo-second-order equation for 24/0 and 96/0. Similarly, the average rate on the pseudo first order and pseudo-second were to 47/0 and 99/0 respectively.

Conclusion: These data show that the better the relationship between the pseudo-second-order adsorption kinetics of pseudo-first-degree biological markers of metals nickel and zinc.

Keywords: heavy metals, nickel, zinc, biological uptake, kinetics, Bubble Tower

P1001: **Kinetics studies removal of heavy metals from industrial wastewater using bacterial biomass**

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2- Chemical Engineering Department, Arak University, Arak, Iran

Background and Aim: Heavy metal ions have been known to be widely discharged into environment and water bodies from industries such as metal plating, pigments, batteries, electrical, tannery, paints, oil refining, and chemical manufacturing. Biosorption, the process of passive cation binding by dead or living biomass, using low-cost biomaterials and microorganisms for removing and recovering toxic metals from industrial wastewaters, has emerged as a potential alternative method to conventional techniques. Biosorption as a biotechnology for removal of heavy metals pollution from aqueous solutions has been extensively studied and most biosorption research mainly focused on the process kinetics.

Methods: Microorganisms were isolated from effluent of an industrial zone of Arak, Iran (Kheir Abad Industrial Zone). Bacterial screening was performed from four different location of the zone. To isolate microbes from sewage, bacteria were isolated through serial dilution method on various media. Few colonies of bacteria due to high metal concentrations were observed. The metal resistant bacteria were isolated and amplified. The amplification was performed using biomass production. For this purpose, a medium of agar was used and then, the colonies were washed from the agar surface and were collected. The bigger colonies were identified with microbiological method and then reproduced. Inoculation rate was selected to be 15% of the volume of wastewater in the reactor. The experiments were done in an internal and external loop airlift bioreactors with and without using packed bed. Biosorption of lead, nickel, zinc and cadmium from industrial wastewater by Bacillus, Pseudomonas, Klebsiella and Escherichia coli was studied in an internal and external loop airlift bioreactor with and without using packeh bed. The pseudo-first-order, pseudo-second-order, Elovich and intraparticle diffusion kinetic models were applied to study the kinetics of the biosorption processes.

Results: Results showed that biosorption is a useful method for removal of heavy metals. Also, isolated bacteria used in this study were capable to adsorption of heavy metals. Conformity between experimental data and models predicted values was expressed by correlation coefficients (R2, values close or equal to 1). A relatively high R2 value indicates that model successfully described kinetics of heavy metal biosorption.

Conclusion: Biosorption of lead, zinc, cadmium and nickel in an internal airlift bioreactor with Bacillus, Pseudomonas, Klebsiella, and Escherichia coli in internal and external airlift bioreactor with and without using packed bed was investigated. In order to investigate the mechanism of biosorption, characteristic constants of adsorption rate were determined at different time by using a pseudo-first-order, pseudo-second-order, Elovich and intraparticle diffusion equation. Biosorption of heavy metals onto bacterial biomass in all bioreactors obey the pseudo-second order kinetics, which suggests that the rate-limiting step of this sorption system may be chemical sorption or chemisorptions involving valency forces through sharing or exchange of electrons between sorbent and sorbate.

Keywords: biosorption, kinetic models, airlift bioreactor, heavy metal,Klebsiella,Escherichia coli

P1002: **Crude oil degradation by bacteria isolated from Bandar Abbas coast and determination of the factors influencing biodegradation**

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Background and Aim: Hydrocarbons present in the marine environment can originate from natural oil seepage and human activities including extraction, transportation, refining, storage, and utilization of petroleum (crude oil). Bioremediation a technology that utilizes microorganism to reduce environmental contaminations has become a popular and effective remediation technique. Although a variety of bacteria that can use crude oil as the sole carbon and energy source have been isolated from the different sites, success in application of bioremediation depends largely on the environmental conditions, such as nutrients, salinity, temperature and pH. The purpose of the present study is to isolated crude oil degrading bacteria and determined factors affecting petroleum consumption by isolated efficient bacteria from Bandar Abbas coastal sediments using Plackett-Burman design.

Methods: Sediment samples were randomly collected at different locations in Bandar Abbas coast. Fresh sediments were transferred into the conical flask containing mineral salt medium with crude oil as sole carbon and energy source. At the end of the enrichment, bacterial strains were isolated by spreading the 10-fold serially diluted on nutrient agar plates. The purified strains were then identified by biochemical and molecular identification (16S rDNA). Nine independent variables with two dummy variables in twelve combinations were organized according to the Plackett-Burman design matrix. The factors tested were: salinity (.05 and 4%), inoculum (0.05-0.6 OD600), temperature (15 and 35oC), pH (9 and 6), time (3 and 7 day), rpm (80 and 140), NH4Cl (0. 2 and 1.7 g/l), K2HPO4 (0. 1 and 0. 5 g/l), and Fe2SO4 (0.005 g/l and 0.04 g/l), the tested concentration range. At the end of experiment remaining crude oil was extracted using n-hexane at pH=2 in each conical flask using separating funnel.

Results: three bacterial strains were isolated on the basis of their growth in mineral salt medium containing crude oil. Among the isolated strains, TA1 was showed the best oil degradation activity. According to morphological, biochemical and 16S rDNA techniques selected strain showed close relation to Alcanivorax sp. (99%). Results showed that main parameters affecting the oil degradation were recognized as time, rpm, NH4Cl, pH, temperature, K2HPO4 and inoculum with main effect 16.15, 11.67, 6.94, 6.47, 4.72, 1,98, and 1,96 respectively.

Conclusion: In our study three bacterial strains isolated from surface aerobic sediments. Among isolated strains, TA1, Alcanivorax sp. showed the best crude oil degradation activity. Microbes such as Alcanivorax provide a major route for the breakdown of oil pollution, moreover they are one of the most important worldwide due to the fact it produces a wide variety of very efficient oil-degrading enzymes. With this knowledge, TA1 could provide a useful tool for bioremediation of oil spills. Our result showed that Plackett-Burman experimental design is valuable tool for the rapid evaluation of the effects of the various medium components.

Keywords: Alcanivorax, Plackett-Burman, Bandar Abbas, Bioremediation

P1003: **Evaluation of L-Lysine amino acid semi industrial production by cloning**

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Background and Aim: L-lysine, one of the essential amino acids, is used as a mediacament, supplement in human diet and feed additive in animal food. Demand for it in different industries has grown research trials for its production methods in recent years.

Methods: In this study, method of L-Lysine amino acid production was optimized by genetic manipulation. After chromosomal DNA extraction from Corynebacterium glutamicum ATCC 21799 and primer designing, the diamino pimalate dehydrogenase (ddh) gene was isolated from genome by PCR. TAcloning and pET28a (expression vector) recombinant vectors were constructed and transformed into E.coli DH5α cells (first) and E.coli BL21(DE3) respectively. SDS-PAGE and end product assay were used to confirm the gene cloning.

Results: Appearance of a 1150 bp band and nucleotide sequencing of recombinant vector, implied that the cloning of target gene was done correctly. Furthermore, observation of recombinant protein band in SDS-PAGE gel and increase of L-Lysine production by recombinant cells were another reasons of correct cloning and enzymatic activity.

Conclusion: In this research for the first time, expression rate of diamino pimalate dehydrogenase gene in pET28a vector was studied. It showed significant increase.

Keywords: L-Lysine amino acid, ddh gene, Corynebacterium glutamicum, genetic optimization

P1004: **Development of cellulase hyperproducing mutants derived from the fungus Trichoderma reesei**

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Background and Aim: Filamentous fungi Trichoderma reesei are considered to be one of the most efficient producers of cellulase capable of degrading cellulose which is the most abundant biopolymer on the Earth. In recent years, cellulases have become industrially important enzymes that are used in a variety of biotechnical applications such as in food, animal feed, pulp and paper, textile and detergent industries and especially in bioethanol technology. In order to make those applications economically feasible, the yields of the cellulolytic enzymes needs to be increased. So investigation on improvement of cellulase productivity is a remarkable object for research. The aim of this study was to enhance extracellular cellulase production of T. reesei using gamma irradiation.

Methods: We obtained the T. reesei strain (PTCC 5142) from the Persian Type Culture Collection and maintained it on PDA(Potato-Dextrose-Agar) plates. Mutants were generated by exposing spore suspension of T.reesei to 250 Gy as optimum dose using the gamma cell 60co located at the Researches of Agronomic, Medic and Industrial Research Institute of Nuclear Science and Technology Research Centre, karaj, Iran. The survivors were grown onto PDA plates and incubated at 28°C for 7 days. 21 mutant strains with better sporulation were picked up out of around 150 mutated spores. Shake flask experiments were carried out in 250-ml Erlenmeyer flasks. Seed cultures were prepared by inoculating 1.0 ml spore suspension of wild-type and mutant strains to 50 ml inoculum medium and incubated at 28 ˚C and 180 rpm for 24 h. To induce enzyme production, the resulting mycelium were transferred into 50 ml of fermentation medium containing 5 g/l walseth cellulose in 500-ml Erlenmeyer flasks and incubated at 28°C, for 48 h with constant shaking at 180 rpm. The culture supernatants were separated from the cellular mass by centrifugation and used as the enzyme sources. The wild type and mutant strains then were tested for their total cellulase activity (FPase) according to the IUPAC (The International Union of Pure and Applied Chemistry) recommendation and the released reducing sugar was determined by dinitrosalicylic acid method using glucose as the standard. All the experiments were performed in three replicates.

Results: According to the statistical significant difference test, in 67% of the mutant strains significant increase ($p<0/05$) in cellulase production was observed. FPase activity of the mutants ranged from 4.810 to 10.0 U/ml, compared with 5.773 U/ml for the wild type. Of the 21 mutants evaluated, the strain No. 8 was found to be the best strain for cellulase production. This mutant produced 10 U/ml FPase which was 1.7 fold higher than that in parent one.

Conclusion: In conclusion, the present work shows that the improvement of cellulase production capability in T. reesei, through induced mutagenesis by gamma rays resulting 1.2 to 1.7 fold increase in FPase, compared with the wild type. These mutants can be further enhanced by additional mutation using different mutagenic agents in combination with medium optimization to increase their cellulase enzymes yields and could be potential candidates for the bioconversion process.

Keywords: cellulase; Trichoderma reesei; mutation; gamma irradiation

P1005: **Strain improvement of Trichoderma viride for increased production of cellulase**

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Background and Aim: Trichoderma species have been shown to secrete large amounts of cellulases which render them attractive strains for industrial production. However, attempts to use their cellulolytic enzymes in the degradation of cellulosic wastes have not been successful because of low enzymatic yields. Hence, several methods including traditional mutagenesis have been employed to improve the activity of cellulase systems in fungi of the genus Trichoderma. The purpose of this study was to obtain gamma induced mutants of Trichoderma viride producing high levels of extracellular cellulase.

Methods: T. viride was isolated from different soils collected from various sources in Iran and maintained on PDA (potato-dextrose-agar) plates and subcultured every two weeks. The spores were harvested from 7-day old PDA plates with saline solution. Then spore suspension was exposed to γ-rays at 250 Gy as optimum dose using the gamma cell 60Co, installed at the Researches of Agronomic, Medic and Industrial Research Institute of Nuclear Science and Technology Research Centre, karaj, Iran. The survivors were grown onto PDA plates and subsequently incubated at 28°C for 7 days. 21 colonies that were superior in sporulation were selected and subcultured 5 times to test their stability. Fermentation experiments were carried out in 500-ml Erlenmeyer flasks with 50 ml of fermentation medium containing 5 g/l walseth cellulose as an inducer carbon source. The flasks were inoculated with 1-day old mycelium and incubated at 28 °C for 48 h with shaking at 180 rpm. After incubation, the cultures were centrifuged and the supernatants analyzed for determination of soluble protein and cellulase activity. Protein concentration was measured based on the Bradford assay using bovine serum albumin as the standard. Total cellulase (FPase), endoglucanase (CMCase) and Avicelase activities were determined by the dinitrosalicylic acid method using glucose as the standard. All the experiments were performed in three replicates.

Results: Of the 21 mutants evaluated, the mutant strain No. "18" exhibited maximum Fpase productivity (93.14 U/mg). This level was 1.9 fold higher than that in parent strain (48.59 U/mg) but the best mutant for CMCase and Avicelase was mutant No. "21", which produced 3 and 2.3 fold increase relative to the respective activities in the wild type (29.79 and 32.56 U/mg CMCase and Avicelase respectively in parent strain and 92.62 and 74.44 U/mg CMCase and Avicelase respectively in mutant No. "21"). However no significant difference ($p<0.05$) in FPase for this mutant and the wild type was observed. The results revealed that the mutant strains No. "15", No. "16", No. "17", No. "18", No. "19" and No. "20" showed more FPase, CMCase and Avicelase activities than the wild type ($p<0.05$).

Conclusion: In conclusion, we achieved efficient cellulase hyperproducing mutants of T. viride with FPase, CMCase and Avicelase of 65–93, 42–93, 40–74 U/mg . The results of this study provide valuable information regarding the use of gamma irradiation to enhance the yields of cellulase enzymes from cellulolytic fungi and their hydrolysis capacity.

Keywords: cellulase; Trichoderma. viride; mutation; gamma irradiation

P1006: **Optimization of Bacillus amyloliquefaciens BEH111,s growth conditions by statistical Placket Burman method for α-amylase production**

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Background and Aim: Amylolytic enzymes are one of the major enzymes used in various industries. Many microorganisms witch use starch as a source of carbon and energy, can produce a variety of amylolytic enzyme.

Methods: In this study, the amylolytic enzyme profile of bacterium. Bacillus amyloliquefaciens BEH111 was determined, and the statistical Placket Burman method was used to establish optimized conditions for the maximum production of α-amylase by this strain. Its enzyme characteristics were also determined.

Results: . Optimization of growth conditions by statistical Placket Burman method showed that tempreture, starch and pepton constraction anh pH had major influence on α-amylase production. The highest level of enzyme produced by this strain was 2000 U/L, that following optimization of producing conditions, reached 4494±9 U/L, which was 2/24-fold higher than produced by an non optimized culture.

Conclusion: The high level of enzyme activity produced by B. amyloliquefaciens BEH 111 can thus be of commercial significance. Furthermore, by having activity at 50oC and above, makes it possible for this enzyme to be applied to processes that use high temperatures. The optimized pH levels of 6-7 that resulted in high enzyme activity can also allow for the potential use of this enzyme in processes such as starch liquefaction and gelatinization.

Keywords:: B.amyloliquefaciens BEH111, α-amylase, Dinitrosalicylic acid, and statistical Placket Burman method.

P1007: **Time stability of yeast alcohol dehydrogenase activity in the presence of polyamines**

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Background and Aim: Yeast Alcohol dehydrogenase (YADH) catalyzes the oxidation of primary and secondary alcohols by NAD+ into the corresponding aldehydes or ketones. The enzymes are used in food, pharmaceutical and chemical industry, synthesis of chiral compounds as well as bioreactors and biosensors. Like other enzyme, YADH activity decreased over time, limiting industrial use of the enzyme. Additives are usually used to compensate for the enzyme reduced activity. On the other hand, Polyamines are known to interact with a variety of biomolecules and critically involve in some important physiological processes. In present study, the effect of polyamines has been investigated on time stability of yeast alcohol dehydrogenase activity at 25°.

Methods: To study the effect of polyamines, reaction mixture contained 10x10-6 Unit of the enzyme solution, 0.01M pyrophosphate buffer (pH 8.5) and polyamines (55 mM for 1,3-diaminopropan, cadaverine, spermidine and spermine, 4mM for putrescine) was incubated at 25°C. Then residual activity was measured over different times with the addition of ethanol (170mM) and NAD+ (1.5mM) and compared with control.

Results: The activity of YADH decreased over time. The results showed that in the presence of 1,3-diaminopropan, cadaverine, spermidine and spermine, the slope of inactivation curve of YADH significantly reduced. But putrescine increased the slope inactivation in compare to YADH in the absence of the additives.

Conclusion: Overall, 1,3-diaminopropan, cadaverine, spermidine and spermine were found to stabilize the enzyme against inactivation over time, while putrescine destabilize the enzyme. Our results propose stabilizing role of polyamines (except of putrescine).

Keywords: yeast alcohol dehydrogenase, polyamines, time inactivation, stabilizing

P1008: **Modulation of Rhizomucor miehei lipase properties via immobilization on nano porous silica particles (SBA-15)**

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Background and Aim: By developing technology and human awareness of chemical catalyst hazardous the demand of using enzymes as catalyst grows. Like the other catalysts enzymes accelerate the reaction by lowering the activation energy [1]. Compared to chemical catalyst they have more selectivity and can catalyze complex reactions in extremely moderate condition[2]. Despite this advantages, enzymes have some disadvantages such as: High cost, instability in presence of organic solvents and low thermal stability. These problems can be overcome by enzyme immobilization techniques. Enzyme immobilization take place via different ways such as covalent bonding, cross linking, hydrophobic interactions, etc [3].

Methods: In this study modified nano silica particles (SBA-15) was chosen as carrier to immobilize Rhizomucor miehei lipase (RML) in order to improve its thermal and co-solvent stability. SBA-15 is a suitable support due to its high adsorption capacity, chemical, thermal and mechanical stability and compared to macro silica particles it has more area available for immobilization[4-7]. To modify SBA-15 surface for enzyme immobilization through covalent bonding, first SBA was functionlized with 3-Glycidoxy propyl trimethoxy silane (GPS) followed by immobilization of RML on it.

Results: Thermal stability of immobilized RML was investigated and compared to the free enzyme. The results showed that immobilization caused to improve stability of RML up to 65 ?C while free enzyme lost its whole activity after 2 hour at 55 ?C. Further investigation on co-solvent stability of the derivative was made in presence of six organic solvents (10% and 20% of DMSO, THF, Acetonitrile, 1-propanol, 2-propanol & Dioxane). Comparison of results from free and immobilized enzyme showed high co-solvent stability for immobilized preparation of RML.

Conclusion: following enzyme immobilization on different surfaces,not only expenses decrease but also enzyme stability increases.As a result of its higher stability,enzyme can be used in higher temprature whithout denaturing.

Keywords: Rhizomucor miehei lipase, immobilization, nano porous silica particles ,SBA-15)

P1009: **Examination of the Effect of Pasteurization for Eradication of Human Pathogenic Bacteria on the Process of Mushroom Composting**

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Background and Aim: During mushroom composting, it is necessary to remove some harmful microorganisms such as human pathogenic bacteria and remain effective and beneficial thermopile microorganisms (simultaneously) together, to prepare it for Agaricus biosporus cultivation or its mycelia stock. This investigation was conducted to obtain optimum temperature for increasing human pathogens bacteria eradication and decreasing effective thermopile bacteria killing during compost pasteurization.

Methods: Marked samples of compost phase I were grown in differential media and some human pathogenic genuses of bacteria like E.coli, Listeria, and salmonella were detected. The samples were then pasteurized during compost phase II by different processes of time and temperature to determine the ideal pasteurization method for mushroom composting.

Results: The samples at the end of the compost phase II (pasteurization) were cultured in LSA, BGA, Endo medium for detection of Enterobacteriaceae and Listeria. Allcultures were negative. The marked samples were cultured in CYM, CGA at different temperatures. Results showed not only the growth of thermophilic Actinomycete and Fungi, but also revealed that growth was most effective at 58.5°C.

Conclusion: A gradual Increase in temperature up to 58.5°C result in the activation of many genuses of thermophilic Fungi and Actinomycete and the omission of pathogenic bacteria. This enables optimum culture for mushroom mycelia growth and further adversely affects harmful microorganisms.

Keywords: Agaricus biosporus , Pasteurization, compost

P1010: **Production an α-Amylase exoenzyme from moderately halophilic bacteria from Joghatai.**

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Background and Aim: Halophilic bacterial are organisms that grow in environment with very high concentration of salts. Moderatly halophilic bacteria have the potential for exciting and promising applications. Many of them produce compounds of industrial interest such as: enzymes, polymers and osmoprotectans

Methods: In the present study, several bacterial strains were isolated from salt river (Kale Shor) of Joghatai. One of them can grow in media with 2-15% NaCl concentrations. The 16S rRNA gene sequence of these strains performed. Morphological, physiological and biochemical characteristics of this strain are studying by optimizing its growth conditions such as pH and temperature. This strain could produce an α-Amylase exoenzyme that maximum production of enzyme will investigate in variety of pH and temperature range.

Results: α-Amylase is an enzyme that acts as a catalyst for the hydrolysis of alpha-linked polysaccharides into α-anomeric products. The enzyme can be derived from a variety of sources, each with different characteristics.

Conclusion: Special properties α-Amylase from Halobacteria is candidate them extensively to use in various industrial processe.

Keywords: Moderately Halophile bacteria, α-Amylase, Joghatai

P1011: **Optimization of glutamic acid production by using Corynebacterium glutamicum PTCC 1532 with Taguchi method**

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Background and Aim: Corynebacterium glutamicum PTCC 1532 was used for enhancing glutamic acid production.

Methods: For this purpose, five different carbon sources (glucose, fructose, sucrose, maltose and galactose) were used and also the effect of different nitrogen sources (yeast extract, malt extract, peptone and urea) as binary combinations was investigated. The production of glutamic acid was examined qualitatively using thin layer chromatography (TLC). Finally, Taguchi method was used to optimize the culture medium.

Results: The results indicated that peptone and yeast extract together were the best nitrogen sources and sucrose as carbon source was the best substrate for glutamic acid production. The strain gave maximum production glutamic acid in a medium containing sucrose (120g/l), yeast extract (1.2g/l), peptone (20g/l), K2HPO4 (0.8g/l) and MgSO4.7H2O (0.075g/l) at 30°C and 127rpm after 48hrs of fermentation.

Conclusion: The paper shows that better yields of glutamic acid can be obtained by using C. glutamicum. Maximum production of glutamic acid was obtained when different carbon and nitrogen sources were used.

Keywords: Corynebacterium glutamicum, glutamic acid, optimization of fermentation conditions, Taguchi method

P1012: **Isolation and characterization of α-amylase produced by a novel thermophilic Bacillus subtilis**

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Background and Aim: Amylases are important enzymes employed in food, pharmaceutical and fine-chemical industries and producing high thermo-stable and thermo-active starch amylases have received great deal of attention because of their perceived technological significance and economic benefits.

Methods: In the present study, two screening program was applied to identify thermo-stable amylase producing bacteria and characterize the amylase produced by the selected strains. In the primary screening program, bacterial strains were isolated from soil samples of different hot locations and incubated at 37°C for 24 h in starch-nutrient agar. To determine the amylase activity, starch digestion was measured using the colorimetric method which used dinitrosalicylic acid (DNS). 45 particular colonies that have the ability to hydrolyze starch were purified and transferred to activation medium and then to fermentation medium. Three strains with more amylase activity were identified by biochemical and morphological characterization. Then 16S rRNA gene sequencing as a molecular marker was done for final confirmation.

Results: N15 is the highest amylase activity producing strain [19.2 U/mL after 24 h] that was identified as Bacillus subtilis based on morphological and biochemical characterization and 16S rRNA confirmation. At first 5min, the obtained showed zero order kinetics and provided the highest activity at 90°C and pH 7.0. At this condition, Km (Michaelis constant) of the enzyme for degradation of starch was 3.36 mg/mL and maximum rate of reaction (Vmax) was 0.177 mM/min. In the absence of additives and after 30 min of incubation at 90°C, amylase obtained from B.subtiliskept 33.4% of its original activity at pH 7.0 and after 1 h it decreased to 9.1% of its original activity, while at 80°C after 30 min of incubation, the enzyme retained 35.7% of its original activity at pH 7.0 and after 1 h, it reduced to 48% of its activity. Half-life of the enzyme was ~23 min at pH 7.0 and 90°C.

Conclusion: Because of tremendous ability of B. subtilis to produce a thermo-stable amylase enzyme at 90°C without additives in a neutral pH, this strain could be strongly recommended for commercial purposes.

Keywords: Thermo-stable, Bacillus subtilis, Amylase, Activity,16S rRNA

P1013: **Optimization of Poly-β-hydroxybutyrate produced by a strain isolated from oil sludge**

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Background and Aim: Plastics are widely used in applications such as in packaging, building materials and commodities. They are non-biodegradable petroleum derivatives considered as environmentally harmful wastes. Poly-β-hydroxybutyrate (PHB) is a biodegradable polyester that synthesized by bacteria as an intracellular material. It is candidates for the development of environmentally friendly plastics.The main problem in the commercial use of these materials for plastic production is their high cost. Finding suitable bacterial strains, utilization of inexpensive carbon sources can reduce some of the costs.

Methods: The PHB-producing bacteria isolated from oil sludge were investigated. Analysis of PHB production was carried out by Sudan Black B staining, spectrophotometric and gas chromatography methods. A strain with the highest rate of PHB production was used to optimize the culture medium by Response Surface Method (RSM), using molasses a cheap source of carbon.

Results: The maximum PHB obtained from molasses sources was 6.62 g/lit. The results of 16S rRNA ribotyping tests lead to identification of the Bacillus coagulans.

Conclusion: The optimum culture medium by statistical design RSM obtained in this experiments gave a basis for further studies in culture conditions with batch or fed-batch cultivation in a bioreactor for large scale production. Finally, our results suggest that B.coagolans is a good candidate for the production of this biopolymer.

Keywords: PHB, Oily sludge, RSM

P1014: **Production of β-galactosidase by Aspergillus niger PTCC 5010 Using Solid State Substrates**

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Background and Aim: β-galactosidase is mainly used to hydrolyse lactose to its components glucose and galactose. Potential beneficial effects on the assimilation of foods containing lactose, as well as the possible technological and environmental advantages of industrial application. It is used for the treatment of milk and its derivatives for consumption by people who have lactose intolerance, prevention of lactose crystallization in frozen and condensed milk products, formation of galacto-oligosaccharides (GOS) during lactose hydrolysis, the reduction of water pollution caused by whey and also for increasing the sweetening properties of lactose. Therefore, the use of β- galactosidase is one of the most promising applications of enzymes to food industries. The aim of this work was to establish optimal conditions for the maximum production of β- galactosidase using solid state substrates by Aspergillus niger PTCC 5010.

Methods: Aspergillus niger PTCC 5010 was obtained from Iranian Research Organization for Science and Technology (IROST). Taguchi design was employed for screening the most significant factors effecting the β- galactosidase production by strain under study. The effect of addition of various solid state substrates such as wheat straw, rice straw and peanut pod, carbon / nitrogen ratio, incubation time and inducer was studied for optimal β- galactosidase production. β- galactosidase assay was done spectrophotometrically using o-nitrophenyl-β-D-galactopyranoside (ONPG) as the substrate.

Results: The results showed that the highest ?-galactosidase activity was obtained 6375.5 U/mg after 96 h. This study showed that wheat straw supplemented with 0.1% (w/v) carbon / nitrogen ratio was suitable for production of ?-galactosidase.

Conclusion: During the recent years, efforts have been directed to explore the means to reduce the enzyme production costs through improving the yield, and the use of either cost-free or low-cost feed stocks or agricultural byproducts as substrate(s) for enzyme production. Our finding showed that combination of rice straw and peanut pod could be used as suitable and low-cost substrate for β-galactosidase production.

Keywords: β- galactosidase production, Aspergillus niger, wheat straw, peanut pod

P1015: **ISOLATION OF SELENITE- AND SELENATE-RESISTANT MICROORGANISMS FROM SELENIUM-CONTAMINATED WASTEWATERS**

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Background and Aim: Selenium (Se), a naturally occurring element, is essential for biological systems at low concentrations and toxic at higher levels. The accumulation of selenium in certain environments requires the development of efficient detoxification processes. Various species of bacteria reduce selenite and selenate to elemental selenium (insoluble and nontoxic) to eliminate the toxic character of this compound. Thus, bioremediation has been considered as an effective means of cleaning up of selenium-contaminated sites.

Methods: In this study, 263 strains were isolated from wastewater samples collected from selenium-contaminated sites in Iran using the enrichment culture technique and direct plating on agar. The disk diffusion method was performed to determine resistance or sensitivity for these bacteria, and they were identified with conventional biochemical tests.

Results: Four bacterial strains designated QWI 1-4 exhibited very high MIC values ranging from 500 to 760 mM for different forms of selenium (selenite and selenate). Also, our study showed utilization of enrichment culture technique in comparing to the direct plating on agar lead to better isolation of selenite- and selenate-resistant bacteria.

Conclusion: These microorganisms could be further used for bioremediation of contaminated sites.

Keywords: Bacterial strains _ MICs _ Selenate _ Selenite_ resistant

P1016: **Preliminary isolation and characterization "heavy metal" resistance moderately halophile bacteria from Cheshme Palangan Chromite Mine**

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Background and Aim: Heavy metals comprise the major part of the elements in the periodic table. Because they can form complex compounds, some heavy metal ions are essential trace elements, but, essential or not, most heavy metals are toxic at higher concentrations. Halophilic bacterial strains were divided into three groups on the basis of NaCl concentration; first was slightly halotolerant / halophilic; second, moderately halophilic and third, extreme halophilic bacteria. In the present study, several bacterial strains were isolated from Cheshme Palangan Chromit mines of Sabzevar region.

Methods: One of them can grow in media with 2-20% NaCl concentrations. The 16S rRNA gene sequence of these strains performed. Morphological, physiological and biochemical characteristics of this strain are studying by optimizing its growth conditions such as pH and temperature in presence of variety of "heavy metals". Also, in order to study biochemical properties its exo and endo-enzymes content investigated.

Results: . One of them can grow in media with 2-20% NaCl concentrations. The 16S rRNA gene sequence of these strains performed.

Conclusion: This preliminary study showed that this resistant to heavy metals strain is a moderately halophile that may be utilized in various industrial applications.

Keywords: Halobacteria, Heavy metals, Chromit mine

P1017: **A moderately halobacteria strain isolated from Salt lake of Qom**

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Background and Aim: Most of the important groups of bacteria are able to live in concentrations up to about 15% salt and that many groups are physiologically active even at much higher salt concentrations. Halophilic bacterial strains were divided into three groups on the basis of NaCl concentration; first was slightly halotolerant / halophilic; second, moderately halophilic and third, extreme halophilic bacteria.

Methods: In the present study, several bacterial strains were isolated from Qom Salt Lake. One of them can grow in media with 2-15% NaCl concentrations. The 16S rRNA gene sequence of these strains performed. Morphological, physiological and biochemical characteristics of this strain are studying by optimizing its growth conditions such as pH and temperature in presence of variety of “heavy metals”. In order to study enzymetic activities of moderately halophilic bacteria, and specially their relation to salt, exoenzymes content, exposed to the external hypersalin concentration was investigated and an α-amylase exoenzyme detected.

Results: The microbial amylases are the most abundantly produced and used in industry, due to their productivity and thermostability. The next aim in this project will study on exoenzymes.

Conclusion: This strain is a moderately halophilic bacteri.

Keywords: Moderatly halobacteria, salt lake of Qom, exoenzyme.

P1018: **The Isolation and identification of a protease produced from a bacterium of frog skin, the extraction, and the determination of its Stability in Organic Solvents**

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Background and Aim: Proteases are among the most important hydrolytic enzymes and account for approximately 60% of total enzyme sales in the world. Microbial proteases have attracted considerable attention due to commercial application of peptide and ester synthesis in media containing organic solvents. The Bacillus has been found to produce solvent-stable proteases with poten¬tial industrial applications. In this study, an attempt has done to isolate a protease produced from a bacterium of frog skin, to extract protease by different organic solvents and also the effect of organic solvents on proteolytic activity.

Methods: The bacteria were isolated by serial dilution method on nutrient agar according to their morphologies. the identification of the bacterium was performed by molecular method and standard biochemical tests. Proteolytic activity of supernatant was determined by Keay and Wildi method with using casein as substrate. The effects of different concentrations of various organic solvents including acetone, ethanol, methanol, butanol, propanol, chloroform, and ethyl acetate on protease activity were investigated. Finally the best concentration of the solvent was determined for protease extraction.

Results: Based on 16s rDNA sequence, morphological and biochemical properties, the isolated bacterium was identified as a new strain of Bacillus pumilus. This strain was able to produce an extracellular organic solvent- tolerant protease. After 30 minutes incubation at 37 °C, caseinolytic activity of crude protease was stabled in all used organic solvents (acetone, ethanol, methanol, butanol, propanol, chloroform, and ethyl acetate). Extraction of the enzyme was performed by all organic solvents ,but the optimum solvent for better extraction was acetone 95%- 99%.

Conclusion: In this study, we isolated B. pumilus producing an protease from frog skin that was extracted by different organic solvents. By purification, the protease could be used as a biocatalyst for organic solvent-based enzymatic synthesis.

Keywords: frog; Bacillus pumilus; Protease

P1019: **Studies on Bacillus subtilis, as Potential Probiotics, on the Biochemical Parameters of Rainbow trout of urmia city iran**

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Background and Aim: In this study Bacillus subtilis is used for control of streptococcosis with the agent of Streptococcus iniae in Rainbow trout.

Methods: The experience was carried out in 2 groups (Control and Treatment) and 3 replicates. In Control group, probiotic was not applied in diet but in Treatment group, Bacillus subtilis was administered in feed at a concentration of 107 cells g -1. In the day of forty five, 0.1 ml intraperitoneally injection of S.iniae with 2×107 cells ml-1 dosage was done for both of groups and were checked for the rest of the survey duration (2 weeks). At the end of the time which took about two months, blood samples were caught for biochemical experiments for realizing the effect of B.subtilis feeding on resistance of fish against S.iniae infection.

Results: After injection of S.iniae, there was no significant difference among Control(C) and Treatment(T) groups considering parameters such as glucose and aspartate aminotransferase ($p>0.05$). But significant difference was seen in the serum total protein, serum albumin, IgM, lysozyme,urea, Alanin aminotransferase and alkaline phosphatase in both groups ($p<0.05$). The serum total protein, serum albumin, IgM and lysozyme were higher in T group and urea, Alanin aminotransferase and alkaline phosphatase were lower in comparison with the control.

Conclusion: The results of the present study indicate that B.subtilis can be used as an agent for the control of streptococcosis in Rainbow trout hatchery and culture farms for decreasing economical disasters.

Keywords: Probiotic; Bacillus subtilis; Rainbow trout;

P1020: **Evaluation column leaching on the removal of inorganic sulfur from coal by Acidithiobacillus ferrooxidans bacteria**

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Background and Aim: The use of coal as a source of clean, non-polluting heating is the favorite of most countries. The techniques used for the removal of sulfur compounds in coal include physical, chemical and biological processes. Biological processes based on degradation of sulfur compounds by microorganisms are performed under mild conditions with no harmful reaction products and the value of coal is not affected. We studied the evaluation Column leaching on the removal of inorganic sulfur from coal by Acidithiobacillus ferrooxidans bacteria.

Methods: Acidithiobacillus ferrooxidans bacteria culture isolated from the acid mine drainage in Hashoni coal Mine in kerman and the study coal was prepared from this mine . The pyritic sulfur removal from coal by Acidithiobacillus ferrooxidans was studied in the column was 60cm high with an internal diameter of 7.5 cm. In the leaching experiments, the column system was comprised of 2/5kg of coal and 2.5L of 9k medium. Column contained one of the fractions ranging from 0.5-12mm. The effect of various parameters, such as pH , rh , ferric iron concentrations on the rate of biodesulfurization in a period of 40 days was studied. Coal before and after leaching was placed XRD experiments.

Results: The result show that if the particle size is less than 0.5mm,penetration of air into the column is low and cement due to and the lack of access of bacteria to pyrite, sulfur removal decreases. Medium of lowering pH and increase the redox potential shows the oxidation is activated by bacteria on coal and ferrous ion concentration changes as a result of bacterial activity on coal was produced and ferrous ion oxide and ferric ion required to oxidize sulfur in coal provides and oxidation pyrite coal increases with increasing redox potential This bacteria with column leaching method can removal 32% from inorganic sulfur in a period of 40 days, for a particle size of 0.5-12m.

Conclusion:: Use of this bacteria isolated from coal mine showed that term adaptation of these bacteria dropped to remove sulfur from coal and also increases the amount removal of inorganic sulfur from coal. These bacteria can be used in coal washing industry to remove sulfur from the coal and due to reduce air pollution by this fossil fuel.

Keywords: coal, Acidithiobacillus ferrooxidans, Column leaching

P1021: **Investigation of the shelf life of packed bread by fermentation with the antifungal strain lactobacillus**

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Background and Aim: Fungal spoilage is the leading cause of economic loss in the baking industry and may also cause serious public health problems due to the production of Mycotoxins.

Methods: In this study, six LAB strains (lactobacillus Lactobacillus plantarum, Lactobacillus sanfranciscensis, Lactobacillus sakei, Lactobacillus reuteri, Lactobacillus fermentum, Lactobacillus delbrueckii and Lactobacillus brevis) isolated from traditional bread in Iran were used to make sourdough. Antifungal activity was evaluated using MRS agar diffusion method LAB strains tested in bread preservation added to rate of 20% of sourdough. Then, breads loaves were surface sprayed (1 ml per 100 g loaf) with a conidial suspension (104 conidia per ml) of penicillum, aspergillus and fusarium. Then they were packed into polyethylene bags and stored at 30 ° C. The bread shelf life was defined as the time (in days) for molds to become visible on the surface of the packaged loaves. Observations were performed daily. For statistical analysis a completely randomized design with factorial experiment with 4 replications were utilized.

Results: . The results showed that sourdough had significant effect ($p \leq 0.05$) on reduction of spoilage and improvement its microbiological shelf life in comparison with control sample. Also, the samples prepared with lactobacillus L. delbrueckii and L.fermentum had the best shelf life (12 days shelf life).

Conclusion: the samples prepared with lactobacillus L. delbrueckii and L.fermentum had the best shelf life (12 days shelf life).

Keywords: lactic acid bacteria, sourdough, fungal infection, shelf life

P1022: **Isolation of polyextremophile haloalkaliphilic chemolithoautotroph sulfur-oxidizing bacteria from Meighan wetland**

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Background and Aim: Spent caustic is a complicated wastewater produced during various gas and oil sweetening processes in refineries and petrochemical plants. Sulfidic spent caustic usually has pH value over 12, salinity about 5-12% wt and sulfide concentration exceeding 2-3%wt. This toxic waste has very high COD due to elevated sulfide concentration and its disposal to environment may cause environmental disaster. Haloalkaliphile sulfur-oxidizing bacteria (SOB) which can thrive in very harsh conditions can be exploited to treat this industrial wastewater. In this study we tried to isolate SOBs from Meighan wetland which is a unique saline lake in Arak state in the middle of Iran.

Methods: In this study, isolation was performed at pH 10, using sodium carbonate and bicarbonate was utilized as carbon source. Different concentrations of sodium thiosulfate (40, 50, 60, 70 and 80 mM) were added to the media as electron donor.

Results: After enrichment and screening process, 20 facultative autotroph strains were purified which could oxide thiosulfate.

Conclusion: Isolated strains are under further investigation for molecular identification and assessing their potential to remove sulfide in the biological treatment of spent caustic wastewaters.

Keywords: sulfidic Spent caustic, haloalkaliphile chemolithoautotroph sulfur-oxidizing bacteria, Meighan wetland

P1023: **Effect of licoric root extract as a carbon source on biomass and monacolin production by Monascus purpureus PTCC5305**

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Background and Aim: Monascus purpureus, a traditional chinese fermentation fungus, is used as a nutural dietary supplement. Among the metabolites of M. purpurus, monacolin K(lovastatin, mevinolin,mevacor) is found to be very important. It has been proven to be cholestrol-lowering drug. Lovastatin, a hypocholesterolemic agent, competitively inhibits the rate-limiting enzyme of cholesterol biosynthesis 3-hydroxy-3-methyl glutaryl coenzyme A (HMG-CoA)reductase. Cultural condition like carbon and nitrogen sources has a significant influence on the yield of monacolins and biomass production as well. In This study, biomass and monacolins production, in control medium and culture media containing licorice root extract with different carbon and nitrogen sources were compared and evaluated.

Methods: Licorice root extract was prepared by soxhlet method. All cultures were made in equal volume with 3 replications and were adjusted at pH=6. A PDA of the 7 days, mycelia blocks(2×2 mm) of M. purpureus was inoculated in culture media. Then, incubated at 30°C for 7 days with shaking at 130 rpm. After 7 days, cell dry weight and monacolin concentration were measured. HPLC system was used for detection of monacolin at 240 nm. All samples were filtered through 0.45 µm before injection.

Results: In This work, we demonstrated biomass and monacolin K production of M. purpureus, in media containing licorice root extract, was higher than control media at submerged fermentation.

Conclusion: Cultural parameters like carbon and nitrogen, play a significant role in monacolin K production by M. purpureus. In this study, licorice root extract was used as a carbon source. In this project, we proved biomass and monacolin K production by M. purpureus, in media containing licorice root extract, was higher than control media at submerged fermentation. The extract is available and inexpensive material, can be used at industrial processes (for production of Monacolin). It is necessary to optimize other cultural condition like pH, temperature, inoculation size and licorice root extract concentration.

Keywords: Licoric root extract, Monascus purpureus, Monacolin, HMG_COA Reductase

P1024: **Production of antimicrobial metabolites produced by soil origin Gram negative bacteria**

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Background and Aim: Bioactive compounds are special products produced by microorganisms. These compounds are divided into two groups; antibiotics and bacteriocins. The purpose of this study was determination of the antimicrobial spectrum of bioactive compounds produced by soil gram negative bacteria

Methods: In total 64 soil samples were collected and analyzed for production of bioactive compounds. Then bioactive compounds producing bacteria were identified using phenotyping and 16SrRNA Gene sequencing methods. Out of all soil samples, two strains of bacteria with potent activity for production of bioactive compounds were isolated. Phenotypic and 16SrRNA sequencing identification of the isolates recognized them as Commomonas spp. The bioactive compounds produced by these bacteria were partially purified and characterized based on their antimicrobial activities at different pHs and temperatures.

Results: The results obtained indicated that the antimicrobial activity of the compounds for both strains was more at pH 7 and temperature 40°C. On the other hand, these compounds showed antimicrobial activity against Bacillus cereus , Staphylococcua aureus , E.coli as well as Candida albicans and Aspergillus niger . However, Pseudomonas aeruginosa was resistant to the both bioactive compounds.

Conclusion: Overall bioactive compounds produced by Gram-negative bacteria showed activity against some pathogenic bacteria as well as fungi and might be considered as alternative therapeutic agents.

Keywords: antimicrobial metabolites- soil Gram negative bacteria -antibiotics - bacteriocins- 16SrRNA Gene-Commomonas spp-Bacillus cereus

P1025: **Introducing Lysinibacillus sphaericus C3-4 isolated from agricultural soil as chitinase producer**

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Background and Aim: Chitin, a β-1→4 linked polymer of N-acetylglucosamine, is an abundant biopolymer being found in the outer shell of crustaceans, in the exoskeleton of insects, and in the cell wall of most fungi and nematodes, etc. Chitin holds great economic value due to its versatile biological activities and chemical applications, mainly in medical and pharmaceutical areas. Chitinolytic enzymes have been widely used in various processes including the agricultural, biological and environmental fields. The aim of this study was to isolation of chitinase producing bacteria from agricultural soil.

Methods: In this study, chitin was provided from shrimp shell wastes, as the carbon source. Samples were collected from agricultural soil of shahid Chamran university. In order to isolate chitinase producing bacteria an enrichment in mineral base medium (NaNO3, 0.3%; K2HPO4, 0.1%;KCl, 0.05%; MgSO4.7H2O, 0.05%; FeSO4, 0.001%) containing 3% colloidal chitin was performed. Samples were incubated at 28°C on a shaking incubator for 7 days. After every 24-h interval 0.1mL sample was inoculated on a solid medium containing colloidal chitin. These samples were incubated at 28°C and observed for zone of hydrolysis around the colonies.

Results: 6 chitinase-producing bacteria were isolated. These bacteria were able to growth and form zone of hydrolysis in this medium. The colony showing maximum zone of hydrolysis of chitin, namely YSF was isolated and later was identi?ed on the basis of morphological and biochemical tests according to the methods described in Bergey's Manual of Systematic Bacteriology. Then according to molecular identification was identified Lysinibacillus sphaericus C3-4. Optimal growth conditions of temperature and pH of YSF in N.B (Nutrient Broth), were 28°C and 7 respectively.

Conclusion: The results suggest that screening agricultural soils is a suitable approach for finding new chitinase producing strains. The isolated bacterium in this survey has a high potency for chitinase production. The main advantage of this strain was extracellular secretion that facilitate its application in industrial enzyme production. The isolated bacterium in this survey can be used as insecticide or fungicide or as a tool for biodegradation of chitinous substances in environment.

Keywords: Chitinase, Chitin, Lysinibacillus

P1026: **Partial purification and biochemical characterization of a thermostable microbial phytase**

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Background and Aim: Phytase is a subgroup of phosphatases which catalyzes the hydrolysis of myo-inositol hexakisphosphate (phytic acid) to inorganic monophosphate and lower myoinositol phosphates. A diet rich in cereal fibers, legumes and soy protein results in an increased uptake of phytate. Phytic acid has been shown to have a strong anti-nutritive effect in animal as pigs, poultry, fish and human. Undigested phytate negatively affects the absorption of Ca2+, Fe2+, Zn2+ and reduction of phosphate content. It also reduces the digestability of proteins and inhibits digestive enzymes. In addition, some isomers of myo-inositol phosphates have been implicated in blood glucose response, lowering of cholesterol, renal stone formation, in the treatment of Alzheimer's disease, multiple sclerosis and certain types of cancer. Phytases could also be used soil amendment and in plant growth promotion. A phytase in which there is a commercial interest should fulfill a series of quality criteria. As the stomach is the main functional site of supplemental phytase, an enzyme should be effective in releasing phytate phosphate in the digestive tract, stable to resist inactivation by acidic pH and heat from feed processing.

Methods: In this study, samples picked up from a hot spring and inoculated in phytase screening medium. Samples were incubated for 2 days at 40ºC in an orbital shaker (180 rpm). Then all samples were inoculated on PSM agar. A clearing zone around the bacteria colony on PSM plates represents extracellular phytase activity. Bacteria with the highest clear halo have been purified on the specific media. Phytase from thermophilic bacteria have been partially purified using ammonium sulphate fractionation followed by dialysis. Phytase activity and phosphate releasing was determined by end point method. Stability and activity of phytase was assayed in temperature range of 30-90 ºC and a pH range of 2-11.

Results: Results showed that the enzyme was active and stable in high temperature, and it,s maximum temperature activity was observed in 60 °C. In addition, this enzyme was stable between pH 2-11, but the optimal pH activity was found to be pH 8.0.

Conclusion: These results indicated that this phytase can be used in wide range of biotechnological applications.

Keywords: purification; thermostable; phytase; activity; biotechnology

P1027: **Molecular Identification ofLactobacillus with Probiotic Potential from Abomasums Driven Rennet**

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Background and Aim: This study purposes isolation and characterization of Lactobacillus from various sources such as dairy products to show Lactobacillus as beneficial bacteria in functional food industry as well as preventive medicine.

Methods: As the complex micro flora of traditional cheese as a highly-consumed dairy product is greatly influenced by the type of cheese manufacturing process and particularly the use of homemade rennet, in this investigation, the abomasum driven rennet was analyzed for the presence of Lactobacilli with probiotic potential.To differentiate beneficial lactobacilli in the preliminary stage, bacterial suspension was enriched and screened towards acid and bile resistance. The isolated bacteria were subjected to morphological evaluation, antibiotic susceptibility assay and antagonistic effects on pathogenic bacteria. For molecular characterization, 16S rDNA gene was amplified and further evaluated by amplified ribosomal DNA restriction analysis (ARDRA) method using the restriction enzyme TaqI. The ARDRA pattern of each enzyme compared to the virtually digested pattern of previously reported lactobacilli.

Results: The isolates were subjected to the random amplified polymorphic DNA (RAPD) and distinctive lactobacilli were sequenced in terms of 16S rDNA region. Co-interpretation of ARDRA and RAPD data clearly revealed three distinct lactobacilli with higher homology to Lactobacillus acidophilus, Lactobacillus planetarumand Lactobacillus fermentum.

Conclusion: Having considered the outstanding result of compatibility with human gastrointestinal system and diversity of microbial population in ruminant, it has been deduced that the homemade rennet could confer new and compatible probiotic strain to traditional cheese.

Keywords: Probiotics, Lactobacillus, ARDRA, RAPD, Rennet

P1028: **Combined influence of inoculation and aeration rate on synthesis improvement of canthaxanthin by the industrial strain Dietzia natronolimnaea HS-1 in a batch bioreactor**

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Background and Aim: The interest in production of natural colorants by microbial fermentation has been currently increased. Canthaxanthin (CTX, β-β´-carotene-4,4´-dione) is a ubiquitous keto-carotene that is of substantial industrial interest because of its widespread applications in nutraceutical, cosmetic, food and feed industries. Dietzia natronolimnaea HS-1 as an aerobe microbe is recognized as a promising producer of natural CTX. To the best of our knowledge, there is no specific study on the effect of various rates of aeration and inoculation on CTX production by D. natronolimnaea HS-1. Therefore, the aim of this work was to optimize the effects of air-flow rate, inoculation intensity and glucose concentration on the improvement of biomass, total carotenoid, CTX production from D. natronolimnaea HS-1 in a batch bioreactor using response surface methodology (RSM). The effects of D-glucose concentration (3.18-36.82 g/l), inoculum size (12.5×109-49.5×109 CFU cells/ml) and air-flow rate (1.95-12.05 l/l min) on the biomass, total carotenoid and canthaxanthin (CTX) accumulation of D. natronolimnaea HS-1 were scrutinized.

Methods: The strain of bacterium D. natronolimnaea HS-1 used in this work was obtained from Bioprocess Engineering Laboratory (BPEL), University of Tehran, Iran. Pure cultures of the strain of D. natronolimnaea HS-1 from the YMA were transferred into 500-ml Erlenmeyer flasks containing 100 ml of a GPY medium (per liter: 10 g glucose, 10 g peptone, 6 g yeast extract) as a pre-culture to inoculate into batch bioreactor for the bioproduction of CTX. pH, temperature and stirring rate were 31°C, 7.0 and 350 rpm, respectively. The levels of factors (glucose concentration (X1), inoculation intensity (X2) and aeration intensity (X3)) and the effect of their interactions on the production of biomass, total carotenoid and CTX were determined through the RSM. The produced biomass and carotenoid contents were analyzed by the method applied by Khodaiyan et al. (2007). A HPLC system with UV–visible detector was also used to determine the CTX content.

Results: Second-order polynomial models with high R2 values ranging from 0.978 to 0.990 were developed for the studied responses using multiple linear regression analysis. The polynomial models for the biomass (Y1), total carotenoid (Y2), and CTX (Y3) levels with the coefficients in actual values were expressed as follows: $Y1=7.71+0.54X1+0.33X3-0.84X12-0.35X22-0.87X32+0.25X1X2+0.29X1X3$ (1) $Y2=5.28+0.50X1+0.15X2+0.12X3-0.66X12-0.45X22-0.33X32-0.25X2X3$ (2) $Y3=4.91+0.38X1+0.21X3-0.70X12-0.45X22-0.40X32-0.13X1X2-0.11X2X3$ (3) The models showed the maximum cumulative amounts of biomass (7.85 g/l), total carotenoid (5.48 mg/l) and CTX (4.99 mg/l) could be achieved at 23.38 g/l of D-glucose, 31.2 × 109 CFU cells/ml of inoculation intensity and air-flow rate of 7.85 l/l min. The predicted values for optimum conditions were in good agreement with experimental data.

Conclusion: The obtained results can be seen as an effective contribution to the development of more efficient bioprocesses for industrial synthesis of CTX. The CTX isolated from D. natronolimnaea HS-1 may be used as a natural antioxidant for possible production of healthy-functional foods in the future.

Keywords: Dietzia natronolimnaea HS-1, Microbial canthaxanthin, Bioreactor, Air-flow rate, Inoculum size

P1029: **Isolation and Identification of Novel actinomycetes effective in Biosorption of Cd from Southern Coast of Caspian Sea (north of Iran)**

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Background and Aim: Pollution in coastal waters is caused by difference sources. The main source of pollutants is heavy metals industrial and domestic wastes. Among heavy metals, cadmium has toxic effects are especially pronounced in animals of higher trophic levels, particularly in humans. Many types of organisms have been studied for their heavy metal uptake capacities, which actinomycete .

Methods: After collecting water samples from different parts of coast between July and september 2011, samples were serially diluted and plated on starch caesin agar (SCA). Filtered sea water was used for media preparation. The plates were incubated at 30 ºC for 7 days and the colonies obtained were purified. Biomass of Actinomycete used for removal of Cd from aqueous solutions in batch mode or shake flask condition. Samples were withdrawn after specific intervals of time for 72 hours and thereafter the samples were filtered with qualitative filter paper. Then after appropriate dilution cadmium analysis was carried out using atomic absorption spectroscopy. Identification of the effective strains was performed by PCR sequencing.

Results: 16s rRNA gene sequence amplified from the genomic DNA were submitted to GenBank databases. After sequencing genes and aligned them with genome sequences available in NCBI by BLAST software, 4 new species of Streptomyces were identified with accession number of JQ228445, JQ228446, JQ004801 and JQ004802, respectively. It was found that Streptomyces sp. barghi2, Streptomyces sp. barghi1, Streptomyces sp. sadati1 and Streptomyces sp. sadati2 were able to biosorp 39.36, 54.65, 29.25 & 17.46% of cadmium respectively. It is clear that among all Streptomyces sp. barghi1 is most active in biosorption of cadmium.

Conclusion: Removal of cadmium from effluents before they are discharged into the environment can be accomplished by chemical processes, are very expensive. Biosorption by inexpensive biomaterials promises to be an excellent alternative. Unlike precipitation, this process does not generate toxic chemical sludge.

Keywords: Marine Actinomycetes, biosorption, Cadmium ion, Caspian Sea

P1030: **Selection, identification and optimization of crude oil degrading bacteria from Kish Island**

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Background and Aim: Petroleum contamination causes significant environmental impacts and presents substantial hazards to human health. In comparison with other conventional clean up technologies, bioremediation has many advantages on the basis of the ability of microorganisms to break down hydrocarbon effluent into non toxic products. Biodegradation of crude oil success depends on the optimal condition for the oil-degrading isolates; however, information on this aspect is still scarce. The present study was conducted to isolate and identify the oil-degrading bacteria from Kish Island costal sediments and to investigate the effect of nine variables in crude oil biodegradation by the best isolated strain using Plackett-Burman design.

Methods: Surface sediments samples were collected from different parts of the coastal area of Kish Island, Iran. Fresh sediments were transferred into the conical flask containing mineral salt medium with crude oil as sole carbon and energy source. At the end of the enrichment, bacterial strains were isolated by spreading the 10-fold serial diluted on nutrient agar plates. The purified strains were then identified by biochemical and molecular identification (16S rDNA). Nine independent variables with two dummy variables in twelve combinations were organized according to the Plackett-Burman design matrix. The factors tested were: salinity (0.5 and 4%), inoculums (0.05 and 0.6 OD600nm), temperature (15 and 35˚C), pH (6 and 9), time (3 and 7day), rpm (80 and 140), NH4Cl (0.2 and 1.6 g/L), K2HPO4 (0.1 and 0.5 g/L), and FeSO4.7H2O (0.005 and 0.04 g/L). At the end of experiment remaining crude oil was extracted using n-hexane at pH=2 in each conical flask using separating funnel.

Results: Total of four bacterial strains were isolated from enriched consortium, using crude oil as the sole carbon and energy source. Among the isolated strain a bacterium which has higher potential on consumption of crude oil, based on morphological characteristics, biochemical tests and analysis of 16S rDNA has been identified. This bacterium belongs to the Halomonas sp. In order to find out the most significant factors affecting crude oil degradation, Plackett-Burman factorial design was applied. Results showed that main parameters affecting the oil degradation were determined as time, rpm, NH4Cl, salinity and temperature with main effect 16.15, 11.67, 6.94, 6.47 and 4.72 respectively.

Conclusion: In our study the four bacterial strains isolated from surface aerobic sediments. Among isolated strains, Halomonas sp. showed the best crude oil degradation activity. Oil degradation of the genus Halomonas sp. have been reported worldwide. Our result showed that Plackett-Burman experimental design is valuable tool for the rapid evaluation of the effects of the various medium components. In the present study, the variables including time, rpm, NH4Cl, salinity and temperature contributed positively. However, FeSO4 7H2O, OD, pH and K2HPO4 contributed negatively.

Keywords: Biodegradation, Oil pollution, Plackett- Burman design, Halomonas sp

P1031: **Characterization of a Novel Organic Solvent Tolerant Protease from a Moderately Halophilic Bacterium and Its Behavior in Ionic Liquids**

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Background and Aim: Microbial proteases are among the most important hydrolytic enzymes which represent one of the three largest groups of industrial enzymes and account for approximately 60%of the total enzyme sales in the world. In present study, we report purification and biochemical characterization of an alkaline protease produced by the moderately halophilic bacterium strain MS-7.

Methods: The Salinivibrio sp.strain MS-7 was isolated from the Maharlou salt lake in Iran. MS-7 protease was purified by a combination of acetone precipitation and ion exchange chromatography on DEAE-Cellulose. By SDS-PAGE it was achieved that it is a low molecular weight extracellular protease as compared to the other proteases that reported up to now. The effect of some ions on enzyme activity was investigated. Stability of the MS-7 protease toward some denaturing reagents and organic solvents was studied.

Results: An extracellular protease was purified from a novel moderately halophilic bacterium Salinivibrio sp. strain MS-7 by the combination of an acetone precipitation (40–80 %) step and a DEAE-cellulose anion exchange column chromatography. Kinetic parameters of the enzyme exhibited Vmax and Km of 130 U/mg and 1.14 mg/ml, respectively, using casein as a substrate. The biochemical properties of the enzyme revealed that the 21-kDa protease had a temperature and pH optimum of 50 °C and 8.0, respectively. The enzyme was strongly inhibited by phenylmethylsulfonyl fluoride, Pefabloc SC, chymostatin, and also EDTA, indicating that it belongs to the class of serine metalloproteases. Interestingly, Ba2+ and Ca2+ (2 mM) strongly enhanced the enzyme activity, while Fe2+ and Mg2+ activated moderately and Zn2+, Ni2+, and Hg2+ decreased the enzyme activity. The effect of organic solvents with different logP on the purified protease revealed complete stability in toluene, ethyl acetate, chloroform, and n-hexane at 10 and 50 % (v/v) and moderate stability even in 50 % of DMSO and ethanol. The behavior of the MS-7 protease in three imidazolium-based ionic liquids exhibited suitable activity in these green solvent systems, especially in 1-hexyl-3-methylimidazolium hexafluorophosphate ([C6MIM][PF6]).

Conclusion: These findings suggest that the protease secreted by Salinivibrio sp. strain MS-7 can be introduced as a candidate for biotechnological applications based on its haloalkaline properties.

Keywords: Salinivibrio, Halophile, Extracellular protease, Organic solvents, protease

P1032: **Isolationof sulfur oxidizing thiobacillus from manganese mine in gom**

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Background and Aim: Occurrence of sallin alkalin and deficint soil has become an important problem in the qom wich has affected important cash crops, also bioleching manganes.sulfur oxidizing thiobacillus was isolated from mine soil in qom.

Methods: . Using thiosulfate enrichment medium and then purifidon solid thiosulfat-nobel. Out of the 5 isolated obtained, 1 was screened based on their efficacy to reduce the pH of the growth thiosulfat medium as an energy source from 8 to ≤ 3. Sulfate is the end product of reduced sulfur compound. the genus utilizing different redused sulfur compound (sulfide, elemental sulfur, thiosufate and sulfit) as cheolitothrophic substrates. The genus was able to use carbon dioxide as carbon source. Strictly aerobic.

Results: The selected isolated was characterized using test of microscopic, macroscopic and biochemical, related to the genus thiobacills.

Conclusion: The geus by the use of mineral media containing elemental sulfur or thiosulfat as energy substrat, the reduced pH medium; as genus thiobacillus can be sutable candidate for bioleching and wastewater treatment.

Keywords: sulfur oxidizing thiobacillus, redused sulfur compound , bioleching

P1033: **Effects of plasma on biofilm formation on medical polymers**

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Background and Aim: The interface between biological systems and engineered organic materials is a key element of biotechnology. Biofilm formation on the surface of medical polymers, especially urinary catheters, is one of the main problem for using in a long time in the body. Many techniques have been presented to reduce the biofilm formation, such as producing new types of biomaterials, biopolymers and surface modification methods. One of the most revolutionary techniques allowing such surface functionalisation is the plasma treatment. This technique presents a vast range of chemical and physical surface modification possibilities. In this work, glow discharge effects on the surface characteristics of the anti-bacterial urinary catheter has been investigated. Plasma can be utilized to alter the surface of catheter by exposing a surface of polymer to non-polymer forming plasma (e.g., N2, Ar etc.) or by depositing very thin layer of plasma polymer on a surface of polymer.

Methods: In the presented study, nitrogen plasma treatment has been used for surface modification of the catheter surface. Plasma was generated in a Pyrex glass tube containing pressure 1.6×10-1 Torr of nitrogen for plasma treatment of a catheter surface. Discharge voltage is about 1.2 kV and current is 150 mA. Urinary catheter placed in the positive Coulomb region of discharge. Then, biofilm formation on plasma treated catheter samples were investigated by cultivation of E. coli in the shaking incubator for 48 h at 37 °C and 100 rpm.

Results: The results showed that biofilm formation decreased about 85% in comparison with pristine samples and its effect was stable at least for a month.

Conclusion: We believe that the plasma treatment causes reactivity to a surface of the catheter through an energy transfer process.

Keywords: plasma, biofilm, medical polymers

P1034: **Isolation of protease-producing thermophilic bacteria from Gheinarche hot spring**

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Background and Aim: The aim of this work was to screen for bacterial isolates from Gheinarche spring. Thermostable enzymes are active and stable at high temperatures and are hence of great technological potential. These enzymes are often isolated from thermophilic microorganisms from natural high temperature environmental such as hot spring.

Methods: Soil and water samples were screened to isolate of thermophilic strains. Skim milk medium was used as selective medium to assess protease production and development of a clear halo zone surrounding the bacterial colonies was evidenced for positive isolates. To produce the enzyme the isolates were cultured in broth medium in a shaker water bath at different temperatures and sampling was performed in time intervals for protease assay. Protease activity by casein assay method and enzyme production curve were determined for each strain.

Results: Among the nine isolates that grew at higher than 65 C, two isolates (strains GF3 and GP4) which producing the most protease in the shortest time is reported as the superior strain. The both strains were rod-shaped, Gram positive, non-sporulating with 0.5 to 0.8 μm width and 2.0 to 6.0 μm length with optimum temperature to growth and protease production at 70C.

Conclusion: Totally, our obtained data revealed that the hot spring can be used as a potential source to isolates the thermophilic microorganisms with potential application in industrial processes under high temperature.

Keywords: Thermostable protease, Gheynarche hot spring, thermophilic microorganism

P1035: **Isolation of biosurfactant producing bacteria from oil activated sludge**

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Background and Aim: Biosurfactants are structurally diverse compounds, essentially produced by hydrocarbon consuming bacteria from cheap and relatively simple substrates. These have greater efficiency and are more effective than chemical forms. They also have less toxicity and are biodegradable in the environment. Biosurfactants are used in various industries including cosmetic and health, food industry, pharmaceuticals and most of all in the oil industry.

Methods: Activated sludge samples were collected from the disposal of petroleum sludge and contaminated soil in Tabriz oil refinery. Samples were enriched by special mediums. Oil spreading technique results in 14 strains, drop breakup test in 19 strains and hemolytic activity of the beta forms with a diameter of more than 1 Cm in 21 strains were positive. After all, among 43 isolated bacteria, 14 strains were screened as biosurfactant producing bacteria.

Results: Emulsification activity tested with different oils. The results showed that UM5, UM8, UM16, UM23 and UM33 strains had 62.5, 60, 60, 62.5 and 60 percent emulsification activities respectively. Foam production was evaluated by factors like bubble type, density and durability of foam and best results were related to UM5, UM8, UM16, UM17 and UM40 strains. Surface tension measurement showed that in 9 strains it was less than 40 mN/m.

Conclusion: To the results of the highly toxicity of chemical surfactants and their costly production we suggest that using this type of bacteria and providing optimal conditions for them, even with their genetically manipulating, we can produce affordable and aggregate surfactants and help the economy by reduction the cost of produce and use of chemical surfactants in different industries.

Keywords: Biosurfactant, Oil hydrocarbons, contaminated soil, Emulsification, Surface tension.

P1036: **Study of antibiotic resistance in *Escherichia coli* and *Staphylococcus aureus* isolates from milk of cows with mastitis.**

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Background and Aim: Mastitis is the most important factor in economic losses in dairy farming industries in the world. *Staphylococcus aureus*, and *Escherichia coli* are the major causes of mastitis. Antibiotics are used in order to control and treatment of mastitis currently. To control and successful treatment of mastitis, its important to detect resistant strains and antibiotics resistance.

Methods: In this study 27 *E.Coli* strains and 17 *S.aureus* stains isolated from milk of cows with mastitis in Mashhad were examined. Bacteriological tests for identification of isolates were performed at the Laboratory of veterinary Medicine in Ferdowsi university of Mashhad. Antimicrobial sensitivity of isolates tested by disk diffusion method using antibiotic disks containing: gentamycin, chloramphenicol, neurofloxacin, trimethoprim-sulfamethoxazole, cephalothin, cephalexin, Kanamysyn and amoxicillin and ampicillin for *E. coli* isolates and 8 disks containing antibiotics gentamicin, chloramphenicol, trimethoprim-sulfamethoxazole, cephalothin, neurofloxacin, oxy-tetracycline, kanamycin and penicillin for *S. aureus* isolates was performed.

Results: in *E.coli* isolates, resistance to the chloramphenicol, oxy-tetracycline and trimethoprim-sulfamethoxazole 22/2%, to the cephalexin 85/1%, to the Kanamycin 29/6%, to the Amoxicillin and ampicillin 100% was reported. In *S.aureus* isolates, resistance to the trimethoprim-sulfamethoxazole and Erythromycin 5/8% and to the penicillin 94/1 % was reported. Overall, among the *E. coli* isolates 14 antibiotic resistance patterns and among the *S. aureus* isolates only 2 antibiotic resistance patterns were found, which shows the variation in multiple resistance in *E. Coli* isolates.

Discussion: widespread use of antibiotics in treatment and control of mastitis increases the risk of creating antibiotic-resistant strains. The results showed high rates of antimicrobial resistance among *E.coli* isolates Than *S.aureus* in mastitis. The identification of strains resistant with antimicrobial susceptibility methods before control and treatment is of great importance in the successful treatment of infected cows. However, the transmission of antibiotic-resistant strains to humans through unpasteurized milk is also important in terms of public health.

Key words: Escherichia coli, Staphylococcus aureus, mastitis, antibiotic resistance

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