

# Evaluation of PCR method for diagnosis of Group B *Streptococcus* carriage in pregnant women

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## Abstract

**Background and Objectives:** Group B *Streptococcus* (GBS) (*Streptococcus agalactiae*) is the leading cause of morbidity and mortality of newborn infants and accounted as a leading factor causing septicemia after birth in mothers. Infections in infants are usually acquired by contact with the genital tract of the mothers during labor and delivery. In two last decades, significant progress toward detection, prevention and treatment of pregnant women carrying GBS has been achieved. A rapid screening test for GBS that could accurately identify pregnant women carrying the bacteria at the time of delivery would obviate the need for prenatal screening.

The standard method for the diagnosis of GBS colonization consists of culturing vaginal and anal secretions in a selective broth medium which inhibits the growth of other microorganisms. Today, it is accepted that PCR has a high sensitivity and specifically in diagnosis. The goal of this study was to screen pregnant woman carrying GBS by PCR.

**Material and Methods:** Samples were taken from anal and vaginal mucus of 125 pregnant women who were at 28-38 weeks of gestation by swab. Samples were tested by standard culture using Todd Hewitt Broth and Blood Agar and also by PCR using primers specific for *cfb* gene.

**Results:** Culture identified 10 (8%) women as carriage of GBS out of 125 women tested. On the other hand, the PCR assay could identify 12 (9.6%) women positive for GBS. In comparison to culture results, sensitivity, NPV, specificity, and PPV of PCR were 100%, 100%, 98%, and 83%, respectively. The time required for PCR assay and culture were 2h and 36h, respectively.

**Conclusion:** We found that GBS can be detected rapidly and reliably by a PCR assay using combined vaginal and anal secretions from pregnant women at the time of delivery. Also this study shows that the rate of incidence of GBS is high in Iranian pregnant women. We, therefore, recommend screening of pregnant women for detecting of GBS emphatically.

**Keywords:** Group B Streptococcus, Pregnant Women, PCR Assay

# The Effect of essential oil of *Matricaria chamomilla* L. on biofilm formation of *Pseudomonas aeruginosa*

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## Abstract

**Background and Objectives:** *Pseudomonas aeruginosa* is an important opportunistic pathogen. The mucoid strains of *P. aeruginosa* produce hyperviscous substances consisting mainly of alginate which have important roles in formation of biofilm. We investigated the effect of essential oil of *Matricaria chamomilla* L. on biofilm production in *P. aeruginosa*.

**Material and Methods:** *P. aeruginosa* 8821M was used as standard strain for biofilm production. Antibacterial effects of essential oil of *M. chamomilla* L. (50% in DMSO) was tested by disk diffusion method. The effect of essential oil on biofilm formation of *P. aeruginosa* 8821M was evaluated following inoculation of bacteria in LB broth medium containing 0.5, 0.35 and 0.2 µg/ml of oil which were incubated for 24h at 37°C. The biofilm formation was measured by Fonseca method. Bacteria inoculated and uninoculated media without oil were used as positive and negative controls, respectively.

**Results:** The results showed that the essential oil did not have any antibacterial effect or reduction in biofilm formation in the presence of 0.35 and 0.2 µg/ml of oil. On the other hand, bacteria biofilm formation was significantly reduced in the presence of 0.5µg/ml of oil in comparison with positive control.

**Conclusion:** This research showed that the essential oil of *Matricaria chamomilla* L. had no antibacterial effect, but caused reduced biofilm formation in *P. aeruginosa*. Biofilm formation is an important virulence factor in mucoid strains and our results may suggest the possible use of essential oil in control of infections caused by *P. aeruginosa* or other related infections.

**Keywords:** *Pseudomonas aeruginosa*, biofilm, *Matricaria chamomilla* L.

# Application of PCR for detection of *Vibrio cholerae* using primers targeted against the gene of outer membrane protein *ompW* and comparison with conventional methods

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## Abstract

**Background and Objectives:** *Vibrio cholerae*, the etiologic agent for the diarrheal disease of cholera, continues to be an important cause of mortality and morbidity in many parts of the world. *V. cholera* serotypes O1 and O139 are associated with classic cholera, however, other *V. cholera* strains, including non-agglutinable vibrios (NAG) are occasionally isolated from the cases of diarrhea. Identification of *V. cholera* is usually achieved through a series of culture and biochemical tests, but close relatedness among *V. cholera* and other member of *Vibrio* spp. or *Aeromonas* spp. has often made identification of the organism quite difficult. The objective of this study was evaluation of PCR targeting outer membrane proteins (*ompW*) for detection of *V. cholera* in comparison with conventional method of culture and biochemical tests

**Material and Methods:** A total of 156 *V. cholera* isolates from both clinical and environmental sources identified on the basis of conventional culture, biochemical tests and serotyping. Polymerase chain reaction (PCR) assay was carried out using primers targeting the gene of outer membrane proteins. Second PCR assay was also performed using primers based on O139-*rfb* region within the *V. cholerae* chromosome.

**Results:** Based on the results from biochemical tests and serotyping, 6 isolates were identified as *V. cholera* O1, serotypes Ogawa (five cases) and Hikojima (one case) and 150 non-agglutinable vibrios (NAG). PCR showed 136 isolates (87.9%) were positive for *V. cholera* and 20 others (12.1%) were negative. PCR results on NAG isolates revealed none of the isolates were belong to O139 serotype.

**Conclusion:** In the present study, PCR assay showed no priority over the conventional methods. The prevalent *V. cholerae* isolates in the region of study were NAG and the least dominant isolates were O1 Ogawa-serotype. No O139 serotype was detected among the isolates.

**Keywords:** *V. cholera*, PCR, Outer membrane proteins, NAG

# Genotyping of *Helicobacter pylori* strains isolated from patients with NUD, DU, GU and GC by RAPD-PCR

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## Abstract

**Background and Objectives:** *Helicobacter pylori* is a genetically diverse gastric pathogen that chronically infects billions of people worldwide, typically beginning in infancy and lasting for decades. It is a major cause of peptic ulcers and it is an early risk factor for gastric cancer which is the most frequently lethal malignancy globally. This project was designed to genotype *H. pylori* isolates isolated from patients with NUD, DU, GU and GC by the polymerase chain reaction (PCR)-based on Randomly Amplified Polymorphic DNA (RAPD) fingerprinting technique.

**Material and Methods:** Eighty patients admitted to the gastroenterology unit at Sharyati hospital in Iran were included in this study. Gastric biopsy specimens were inoculated onto selective medium then were cultured for 3 to 5 days at 37 °C under microaerobic conditions. Genomic DNA was extracted using a commercially available Qiagen kit. RAPD-PCR was used to genotype isolates.

**Results:** Six different RAPD patterns (A-F) were seen in more than one isolate which were as follow; pattern A: 9 (16.98%), B: 6 (11.33%), C: 5 (9.43%), D: 3 (5.66%), E: 2 (3.77%) and F: 2 (3.77%). Twenty six (49.06%) of 53 isolates showed a unique RAPD pattern that were not similar to each other. A significant relationship was not seen between a single RAPD pattern and a gastric disorder ( $P>0.05$ ).

**Conclusion:** The results of this study suggest a high level of DNA sequence diversity among *H. pylori* isolates and it is better to use sequencing method for surveying of *Helicobacter pylori* genome rather than RAPD-PCR.

**Keywords:** *Helicobacter pylori*, RAPD-PCR, Genotyping

# Evaluation of wide broad spectrum antibiotic resistance of *E. coli* and *Klebsiella pneumoniae*

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## Abstract

**Background and Objectives:** The Study of *E.coli* and *Klebsiella* resistance to wide spectrum antibiotics and molecular evaluation of resistance in these bacteria were examined. Treatment with wide spectrum antibiotics against bacteria can lead to resistance. The antimicrobial resistance can be seen in two types: 1) chromosomal alterations which could result in changes in structure of receptors of specific drugs. Most of this mutation can cause the absence of a penicillin binding proteins (pbps) and 2) is plasmid resistance.

Plasmid genes can usually produce enzymes which result in destruction of antibiotics. An example is extended spectrum B-lactamase (ESBLs) which causes resistance to the third generation of cephalosporin, monobactams and new penicillin. ESBL plasmids are derived from TEM-1, TEM-2 and SHV-2. It has been recognized that because of point mutations in these plasmids, there are about 90 types of TEM and 25 types of SHV. The reason for the formation of point mutations within the ESBL plasmids is due to high consumption of broad spectrum antibiotics.

**Material and Methods:** In a cross-sectional study, 218 specimens from patients were collected and after identification of bacteria, the percentage of ESBLs among the isolates was calculated. For this purpose combined disk method and double disk method are used. Then TEM and SHV plasmids from 23 specimens of *E.coli* and *Klebsiella* were examined using plasmid extraction kit and PCR.

**Results:** The result of this study showed; the antibiotic resistance in 10% of *E .coli* was chromosomal and 50% were plasmids. The remaining isolates were sensitive. In *Klebsiella*, 12.8% of resistance was chromosomal and 62% were due to plasmids and remaining isolates were sensitive. The results from PCR showed; in *E .coli* 52.8% of the isolates were TEM positive and 84.6% were SHV positive and 69.2% were positive for both TEM and SHV. For *Klebsiella* 80% were TEM positive and 80% were SHV and 60% were positive for both TEM and SHV.

**Conclusion:** The rate of ESBLs in Iran is higher than the results in similar studies in other countries. This could be because of the overuse of third generation of cephalosporin. For the purpose of having proper treatment using antibiotics, medical education and laboratory detection of ESBLs could be effective to choose choice antibiotics.

**Keywords:** E ESBLs, *E.coli*, *Klebsiella*, PCR

# The level of antibody against *Chlamydia trachomatis* among patients with genital infections in Mashhad

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## Abstract

**Background and objectives:** *Chlamydia trachomatis* is a common cause of sexually transmitted disease which can cause severe consequences. Appropriate preventive requires knowledge of epidemiology of infection in different population in order to target interventions in a cost-effective manner. In this study prevalence of *C. trachomatis* infections were determined according to some parameter in Mashhad.

**Materials & Methods:** In this study serum from 76 patients with STD were examined by ELISA and IFA for *C. trachomatis*. Statistical evaluation was done using SPSS program.

**Results:** ELISA showed that 11 and 3 patients with IgG and IgM against *C. trachomatis*, respectively. IFA analysis showed that 1 patient had titer of 1/32, 6 patients with 1/64 and 3 patients with 1/128. One female patient showed titer of 1/256.

**Conclusion:** This study provides strong evidence that *Chlamydia* prevalence in our region is significantly high which necessitate screening and treatment. It is, therefore, suggested that detection test for chlamydial genito-urinary infections become a routine part of STD investigations.

**Keywords:** *Chlamydia trachomatis*, Seroepidemiology, STD infection.

# Study of spontaneous bacterial peritonitis etiologic agents and determination of their antibiotic resistance pattern

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## Abstract

**Background and objectives:** Spontaneous bacterial peritonitis (SBP) is a frequent and often fatal complication of ascites without a demonstrable intra abdominal cause. In adults, the organisms of SBP are usually gram-negative bacteria, but they may differ in children. Since these organisms are resistant to most antibiotics, identification of active antimicrobial agents and determination of resistance pattern are essential. The aim of the present study was also to determine the causative agents of SBP in children with liver disease and ascites, referred to pediatrics ward of Imam Khomeini hospital during 1384-85.

**Material and Methods:** In this study, ascite samples were taken from 85 patients with liver disease and ascites of Emam Khomeini Hospital, pediatrics ward, and they were examined by direct test, culture on different media and biochemistry tests. Antibiogram tests by disk diffusion were done on each positive sample.

**Results:** Of 85 examined samples, 32 bacterial and 2 yeast agents were isolated. Of bacterial cases, *Escherichia coli* (31.25%) and coagulase negative *Staphylococci* (18.75%) were the most isolated agents and the rest, included *Streptococci* and *Enterobacteriaceae*. Moreover, antibiogram tests identify that most of coagulase negative *Staphylococci* isolates as resistant to cotrimoxazol, amoxicillin, penicillin and cephalosporin (first generation). The most of gram negative isolated bacteria were resistant to amikacin, vancomycin and gentamicin.

**Conclusion:** Since the spontaneous bacterial peritonitis is not detectable by clinical signs, ascite samples should be examined in order to determine the etiologic agents. In general spontaneous bacterial peritonitis agents are mostly composed of normal flora bacteria, in our study most isolated bacteria were *Escherichia coli* and coagulase negative staphylococci, two major normal flora of gastrointestinal tract and skin. The isolated bacteria showed a high antibiotic resistance against common drugs in our study. In general, this study showed that the major agents of spontaneous bacterial peritonitis should be identified by ascite examination and antibiogram test to establish a perfect treatment pattern in order to treat the patients rapidly.

**Keyword:** spontaneous bacterial peritonitis, antibiotic resistance and Disk diffusion

# Antiviral effect assay of twenty five species of various medicinal plants families in Iran

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## Abstract

**Background and Objectives:** Medicinal plants have been traditionally used for different kinds of ailments including infectious diseases. There is an increasing need for substances with antiviral activity since the treatment of viral infections with the available antiviral drugs often lead to the problem of viral resistance. There is a need to search for new and more effective antiviral agents, therefore in the present study 25 plants with ethno-medical background from different families were screened for antiviral activity against HSV-1, Adenovirus type 5, Echovirus type 11, Measles virus and Rotavirus.

**Material and methods:** Different parts of the plants collected from Iran were extracted with aqueous solvents to obtain crude extracts. These extracts were screened for their cytotoxicity against Vero, BSC-1, Hep-II and RD cell lines by micro-culture neutral red dye absorption and microscopically follow up for CPE. Antiviral properties of the plant extracts were determined by cytopathic effect inhibition assay and plaque reduction assay.

**Results:** Four plants extract; *Nymphaea alba*, *Rhus coriaria* L., *Chelidonium majus* and *Terminalia chebula* Retz exhibited significant antiviral activity against HSV-1 and adenovirus type 5 at non-toxic concentration. The extracts of *Chelidonium majus* showed great anti viral activity against HSV-1 and partial activity against adenovirus at higher concentrations.

**Conclusion:** Some of the medicinal plants have shown antiviral activity. Further research is needed to elucidate the active constituents of these plants which may be useful in the development of new and effective antiviral agents.

**Keywords:** Medicinal plants, Antiviral activity, Herpes Simplex virus, Adenovirus

# Correlation of high viral BK load in the urine samples of patients with graft-versus-host with haemorrhagic infectious diseases of bladder after hematopoietic stem cell transplantation

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## Abstract

**Background and Objectives:** A possible temporal correlation between high BK virus (BKV) load in urine alone or in combination with acute graft versus host disease (GVHD) and the development of hemorrhagic cystitis (HC) was examined in this study.

**Material and Methods:** 31 allogeneic hematopoietic stem cell transplanted (SCT) patients were included in this study. BKV DNA was detected by nested and quantitative Real-Time PCR in the urine of 16 out of 31 patients. HC occurred in 6/16 patients with BKV DNA in their urine samples. BKV load was evaluated in the urine samples from 5 of 6 HC patients.

**Results:** Presence of BKV or BKV load  $>10^6$  copies alone in urine samples showed some predictive ability for HC, while acute GVHD alone or conditioning regimens did not. However, during the period after SCT to HC onset a combination of BKV load  $>10^6$  copies and acute GVHD, discriminated the best between HC (4/5) and non-HC (2/25) patients ( $p=0.003$ ).

**Conclusion:** This study indicates that BKV DNA and particularly  $>10^6$  BKV copies/ $\mu$ l of urine from SCT patients may have some predictive ability for HC. However, the best association to HC was achieved when a viral load of  $>10^6$  BKV copies/ $\mu$ l of urine was present in combination with acute GVHD.

**Keywords:** BKV, Urine, Hematopoietic cells, Graft-Versus-Host

# Report on Clonal Dissemination of a New *Vibrio cholerae* Serotype O1 Biotype El Tor Strain in Kurdistan in Summer of 2007

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## Abstract

**Background and Objectives:** Cholerae disease caused by toxigenic *V. cholerae*, is a major public health problem in developing countries including Iran. Epidemiological surveillance and comparative molecular analysis of isolates have demonstrated clonal diversity among epidemic strains and a continual emergence of new clones of toxigenic *V. cholerae*.

**Material and Methods:** A total of 20 *V. cholerae* strains were sent to Pasteur Institute of Iran in September of 2007 from Kordestan province which includes strains of different sub-serotypes. After biochemical identification, isolates subjected to molecular analysis including Pulsed-Field Gel Electrophoresis (PFGE) of *NotI* digested genomic DNA according to the standardized protocol by Centre of Disease Control (CDC).

**Results:** PFGE results showed a single pattern for all isolates.

**Conclusion:** The results were interpreted in comparison with patterns obtained by isolates of previous years and showed clonal dissemination of a new clone in Kordestan province in this year.

**Keywords:** *Vibrio cholerae*, Kurdistan, PFGE, Inaba, 2007