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In the name of God

Iranian Journal of Medical Microbiology
The official publication of the Iranian society of microbiology

Vol.1, No.1, Spring 2007
Quarterly

ISSN: 1735 - 8612

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Evaluation of indirect fluorescent antibody assay for detection of *Nocardia*

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ABSTRACT

Background and Objectives*:* Nocardiosis is an acute or suppurative chronic disease caused by an aerobic, gram-positive, weakly acid-fast and soil-borne filamentous and organism. *Nocardia asteroides* which is the dangerous and most frequently pathogen, infects humans through the respiratory tract. The bacterium is primarily an opportunistic pathogen that causes the infection in patients with underlying immunodeficiencies.

The main purpose of the study was to detect antibody titre against *Nocardia* in all study groups, using indirect immunofluorescent assay (IFA). Correlation between the antibody titre against *Nocardia* with age, sex, occupation, and chronic pulmonary infection and corticosteroid therapy patients was also investigated.

Material and Methods: The present investigation is a *Cross–Sectional* study conducted on a population consisted of 300 subjects including 200 hospitalized individuals' patients, nurses and healthcare workers from Imam Khomeini hospital, and 100 health adult blood donors. None of the patients had already been diagnosed to be affected by Nocardia.

Results*:* Our results demonstrated four patients suffering from different infections, including TB, mycetoma, chronic pulmonary and chronic obstructive pulmonary diseases were IFA positive. None of the high risk hospital personnel, who were working in close proximity to the areas infected with *Nocardia*, were found to be IFA positive. Meanwhile there was no positive result in a group of patients (n=34) who were under corticosteroid therapy.

Conclusion*:* Finally, considering the small sample size of the IFA positive cases no significant association between the IFA results and age, sex, occupation and clinical conditions of the subjects could be established.

Keywords: Nocardiosis, *Nocardia asteroides*, immunodeficiencies, Indirect immunofluorescence assay (IFA), Chronic obstructive pulmonary disease (COPD).

Aminoglycosides modifying enzymes genes among the population of enterococci in Tehran

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ABSTRACT

Background and objectives: Resistance to high level concentration of gentamicin is widespread among isolates of enterococci at Tehran Hospitals. To understand the mechanism of resistance among the Iranian isolates, we screened a collection of *E. faecalis* and *E. faecium* isolates to detect aminoglycoside modifying enzymes genes.

Material and Methods: To detect the high level gentamicin resistant isolates of enterococci (HLGR phenotype, MIC>500 µg/ml), 114 clinical isolates of *E. faecalis* (n=79) and *E. faecium* (n=35) were tested with disks containing 120 µg of gentamicin. The macrobroth dilution assay was then used to determine the minimum inhibitory concentration of gentamicin. The susceptibility of isolates against amikacin, netilmicin, tobramycin, kanamycin were also determined by Kirby-Bauer method. All isolates were subjected to polymerase chain reaction assays targeting aminoglycoside modifying enzyme (AMEs) genes including *aac(6′)-aph(2″) , aph(2″)-Ib, aph(2″)-Ic, aph (2″)-Ia, aph (2″)-Id. aph(3′)-IIIa* and *ant(4′)-Ia*.

Results: All isolates with HLGR phenotype and those showing 64<MIC<500µg/ml contained *aac(6′)-aph(2″)*. The *aph(3′)-IIIa* was found among 61% and 65% of isolates with HLGR phenotypes and those with MIC<500 in respects. Co-existence of *aac(6′)-aph(2″)* and *aph(3′)-IIIa* gene among HLGR isolates of *E. faeclais* and *E. faecium* were 59.5% and 64.7% respectively. The gene *aph(2″)-Ic* was amplified in two isolates of *E. faecium*. The results of PCR for *aph (2″)-Id, ant(4′)-Ia* and *aph(2″)-Ib* genes were negative.

Conclusion: The *aac(6′)-aph(2″)* was the most frequent gene encoding resistance to gentamicin and other aminoglycosides followed by *aph(3′)-IIIa*. Isolates lacking these genes were susceptible to all aminoglyocosides used in this study.

Keywords: Aminoglycoside modifying enzymes, Enterococci, HLGR, Gentamicin resistance

Characterization of *rpoB* mutations in rifampin-resistant isolates of *Mycobacterium tuberculosis* cultured from the Iranian patients

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ABSTRACT

Background and objectives*:* In this project we investigated the frequency of mutations within the rifampin resistance-determining region (RRDR) of *rpo*B gene to determine whether this region is useful for molecular detection of rifampin resistant isolates of *Mycobacterium tuberculosis* from Iranian patients.

Material and Methods*:* A set of 25 rifampin resistant and 5 randomly chosen fully susceptible *M. tuberculosis* complex strains obtained from sputum samples of individual patients were investigated. The *M. tuberculosis* H37RvT and CDC 1551 standard strains were used as controls. Using the specific primers, the entire RRDR of rpoB gene of selected samples was amplified and sequenced directly.

Results*:* Genetic alterations in the RRDR were present among 96.0% of isolates. The majority of rifampin resistant isolates (72.0 %) showed missense mutations in the core region of *rpo*B that led to substitutions of amino acids at Ser-531 (60.0 %), His-526 (16.0 %) or Asp-516 (8.0 %). While the codon 531 has been the most common site of nucleotide substitutions worldwide, the frequencies of mutations at the codons 526 and 516 among the Iranian isolates were different from other geographical regions. Mutation at codon 533 was found at higher frequency (8%) comparing to the report from other countries.

Conclusion*:* The high rate of mutations within the RRDR of the *rpo*B gene suggests that targeted screening of the RRDR may be feasible for the determination of rifampin resistance in clinical isolates of *Mycobacterium tuberculosis* from Iran.

Keywords*:* *Mycobacterium tuberculosis*, rifampin resistance, mutation.

Isolation of *Pseudomonas aeruginosa* strains producing metallo beta lactamases from infections in burned patients and identification of bla_{IMP} and bla_{VIM} genes by PCR

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ABSTRACT

Background & objectives: *Pseudomonas aeruginosa* is one of the most important causes of nosocomial infections particularly in immunodeficient patients. Several antibiotics such as betalactams, aminoglycosides and quinolones are used for the treatment of infections caused by this organism, but the prevalence of multidrug resistant strains has been reported frequently. Beta lactamase production is one of the most important resistant factors. Metallo-beta-lactamase (MBLs) have a broad-substrate spectrum, they hydrolyse all beta-lactams except for the monobsctam aztreonam. In this study, the metallo-beta-lactamase production in *Pseudomonas aeruginosa* strains were investigated.

Material and Methods: Initially, the antibiotic resistance pattern of 100 clinical strains isolated from infections in burned patients in Ahwaz Taleghani hospital was determined by disc diffusion method. All the clinical *Pseudomonas aeruginosa* isolates nonsusceptible to imipenem were screened for production of MBLs by Etest with imipenem/ imipenem plus EDTA (Etest MBL).DNA was extracted from colonies by simple boiling method. The extracted DNAs were then examined by PCR involving specific primers for blaVIM and blaIMP MBL genes.

Results: Based on the obtained results, the percentage of resistance was as below: Cefepime100%, Ceftazidime81%, Ticarcillin70%, Imipenem41%, Meropenem23% and Piperacillin20%. The investigation for MBL production in the strains resistant to IPM by Etest MBL showed that 8 out of 41(19/51%) Imipenem resistant isolates were MBL producers. All the clinical *P. aeruginosa* isolates that were nonsusceptible to IPM were then examined by PCR for the presence of the blaVIM and bla IMP genes. Of the 41 *P.aeruginosa* strains isolated during the study period, 8(19/51%) were positive for blaVIM gene.The results from PCR assay represented thet 8 isolates among 41 IPM resistant strains of *Pseudomonas aeruginosa* were positive for blaVIM gene. The remaining 33(80/49%) were negative for VIM and IMP MBLs.

Conclusion: The results of this investigation in agreement with shows that, the antibiotic resistance of *Pseudomonas aeruginosa* is increasing in burn hospitals and the MBLs production can be the cause of this issue.

Keywords: *Pseudomonas aeruginosa*, antibiotic resistance, metallo-beta-lactamase.

An investigation *of aac(6')-le-aph(2")-la* gene in MDR and HLGR *Enterococcus faecalis* and *E. faecium* strains isolated from clinical samples

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ABSTRACT

Background: Enterococci are important nosocomial pathogens. Multiple drug resistance (MDR) is common among Enterococci and presents difficulties for treatment. High level gentamicin resistance(HLGR) in enterococci, is a significant therapeutic problem. Bactericidal antimicrobial activity usually is obtained by the synergistic combination of a cell wall active agent such as penicillin or glycopeptide with an aminoglycoside.

Enterococci can acquire aminoglycoside resistance genes that mediate production of aminoglycoside – modifying enzymes, which eliminate this synergistic bactericidal effect. The most clinically important HLGR genes is aac(6')-le-aph(2")-la.

Material and methods: In the present study , a total of 437 clinical samples from 5 hospital and 3 private laboratory in Tehran , from azar 1384 to Tir 1385 , were collected and 300 enterococcal isolates recovered all of the strains were identified to the species level by conventional biochemical tests and assayed for their susceptibility to 11 antibiotics, ampicillin, tetracycline, erythromycin,ciprofloxacin, high dose gentamicin,vancomycin,cotrimoxazole, quinopristin –daifopristin (synercid), linezolid , teicoplanin and nitrofurantoin by disk diffusion method. Gentamicin MIC was accomplished for HLGR strains.

Results: The most frequent species was *E. faecalis* (81.3%) and then *E. faecium* (18.7%). MDR strains were detected in 50% and 95% of *E.faecalis* and *E.faecium*, respectively. The number of HLGR strains for *E.faecalis* and *E.faecium* were found to be 19.5% and 23.5% , respectively. All HLGR strains showed MIC>1024 µg/mL The PCR results showed that 83% and 100% of *E.faecalis* and *E.faecium* strains carried aac(6')-le-aph(2")-la gene as detected by PCR.

Conclusion: The present study indicates high rate dissemination of aac(6')-le-aph(2")-la gene, suggesting the possible mechanism of transfer of gentamicin resistant genes within the enterococcal population and in this case probable need to new aminoglycosides or other antibiotics would be predictable.

Keywords: Enterococci, HLGR, MDR

Comparison of antimicrobial activity of Respitol-B with mentofin containing menthol, eucalyptus oil

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ABSTRACT

Background and objectives: Some essential oils and their main components have antibacterial activity and used as an antiseptics. Respitol-B (Barijessence, Kashan, Iran) which is similar to Mentofin, an imported antimicrobial agents, contains menthol and eucalyptus oil. Both products are used as disinfectant in poultry farms following vaccination of broilers and pullets. In this study, the antimicrobial activity of Respitol-B and Mentofin, against bacteria, fungi and yeast was investigated.

Material and Methods: The antimicrobial activities of eucalyptus oil and mentho was also tested separately using disk diffusion test and macrobroth dilution assay.

Results: The gram-positive bacteria, yeast and fungi showed more susceptibility to Respitol-B than the gram negative bacteria. Antimicrobial activity of Respitol-B was the same as mentofin. Menthol is more potent than the eucalyptus oil. It enhanced the antimicrobial activity of Respitol-B. However *P. aeruginosa*, *E. col*i and *S. typhi* showed resistance to this compound. Eucalyptus oil had antimicrobial effect against *V. chlerae,* A. *flavus,* and *S. aureus* but had no effect on other tested microorganism

Conclusion: Respitol-B, a compound formulated by Barij Essence shows the same an antiseptic property as Mentofin and can be used for sanitary protocols in poultry farms.

Keyword: Respitol-B, Eucalyptus oil, mentofin, antimicrobial activity

Detection of Mycoplasma DNA from the sperm specimens of infertile men by PCR

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ABSTRACT

Background and Objectives: Infections in accessory sex glands are considered as potential hazards to male fertility. These infections can affect different sites of the male reproductive tract such as the testis, epididymis and male accessory sex glands.Transmission of these infections to female partners causes genital infection, infertility and abortion. The aim of this study was to detect Mycoplasma, as one of the causatives, from the semen of infertile males using PCR assay.

Material and methods: The survey considered 100 infertile men who referred to clinics and had not used antibiotics for 7 days prior to sampling. The infertility of cases was confirmed by a physician specialist. The sperm specimens were collected in sterile condition and sent to the laboratory rapidly. Specimens were examined for presence of *Ureaplasma urealiticum* and *Mycoplasma hominis* by PCR. Meanwhile, the history of vaginal infections and abortion in the female sexual partners was investigated. The results of multiplex PCR were compared with spermogram. All patients had no symptoms of genital infection.

Results: Of 100 infertile men , 33 (33%) were positive for CMU organisms (*Chlamydia, Mycoplasma* and *Uureaplasma*). *Uureaplasma urealiticum* and *Mycoplasma hominis* were detected in 17 and 3 of patients respectively.

Conclusion: Due to some problems in culturing of CMU organisms, PCR can be used as a diagnostic technique to detect such pathogens from seminal fluid of infertile men that leads to choose appropriate therapy in a shortest time.

Keywords: Mycoplasma, infertility, PCR

Titration of specific antibodies to *Mycoplasma pneuomoniae, Chlamydia pneumoniae* and *Legionella pneumophila* among Iranian pilgrims' sera during Hajj season in 2004

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ABSTRACT

Background and objectives: Respiratory tract infection is the most common diseases among Iranian pilgrims during Hajj season. To understand the possibility of bacterial involvement in such infections, we screened the pilgrims' sera to determine the titer of antibodies against *Mycoplasma pneuomoniae* (MP) , *Chlamydia pneumoniae* (CP) and *Legionella pneumophila* (LP) .

Material and Method: Serum samples from 128 pilgrims were collected, before the trip and one month after returning home. Antibodies to MP, CP, LP were assayed using Immunoflourecent and ELISA methods.

Results: IgM antibody titre to CP did not elevated, but IgG antibody titer was increased in 34.58% (n=48) and 15.82% (n=22) of cases, indicating of recent infection. The specific antibodies to MP and LP were not increased.

Conclusion: In pilgrims infected with an atypical respiratory pathogen, *C. pneumoniae* should be considered as an important causative. The true prevalence of this pathogen should be investigated since it relies on the sensitivity and specificity of currently available diagnostic methods.

Keywords: *Mycoplasm pneuomoniae, Chlamydia pneumoniae* and *Legionella pneumophila* antibodies, pilgrim, respiratory infections, antibody titration

Advatages of Rapid diagnosis of Bacterial Meningitis By PCR in compare with Direct Microscopy and culture

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ABSTRACT

Background and Objectives: Acute bacterial meningitis has remained an important cause of death and neurological damages among survivors. Rapid diagnosis of bacterial meningitis is crucial for the early targeting of antimicrobial therapy. The aim of this study was to develop and appply a PCR assay for rapid diagnosis of meningitidis and to compare the results with those obtained by conventional bacteriology.
Material and methods: We assessed 150 cerebrospinal fluid (CSF) specimens from suspected patients by PCR targeting *16S rRNA* gene with specefic primers for *Neisseria meningitidis, Sterptococcus pneumonia* and *Heamophilus influenza*. All speciemns were also examined by conventional bacteriology.
Results: The rapidity of diagnosis increased when bacteriological methods were combined with PCR. Of 150 speciemens tested, 10 were positive for *Neisseria meningitidis* in PCR. Direct microscopy and bacterial culture found 5 and 8 cases infected with this organism respectively.
Conclusion: PCR was more sensitive than direct microscopy and culture for detection of *Neisseria meningitidis*. However, direct microscopy may provide evidences for the quality of specimens and presence of other organisms in the samples. Wet- mount direct microscopy showed morphology and arrangements of the observed organisms that may be helpful in presumptive identification of certain bacteria such as gram negative bacilli and cocci.. Moreover, the observed organisms may be useful in correct selection of culture media in the laboratory and prescription of appropriate therapy by physicians in a quickest time.
Keywords: CSF, Bacterial meningitis. Rapid Diagnostic, and Multiplex PCR