Using of multilocus enzyme electrophoresis to study the heterogenicity of clinical isolates of *Mycobacterium kansasii*

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Abstract

Background and Objectives: Multi locus enzyme electrophoresis (MEE) has been proved to be a powerful technique in population genetic studies and molecular epidemiology of pathogenic micro-organisms. In this study MEE was used to determine the genetic relationships of *M. kansasii* strains cultured from patients at Pasteur Institute of Iran.

Material and Methods: 21 isolates of *M. kansasii* (9 isolates from Iran and 12 isolates from other countries) were analyzed for 12 enzymes loci by MEE. Isolates were grown on LJ slants and BACTEC 13A. The cells were sedimented by centrifugation and their lysates containing the enzymes were extracted by sonication. The horizontal starch gel electrophoresis was used for visualization of enzymes after staining the gels with substrate in solutions or agar overlay.

Results: A considerable genetic diversity was found at different loci of *M. kansasii* suggesting the existence of different sub-species for this organism. It also showed the inaccuracy of some biochemical test for identification of some isolates with in this species.

Conclusion: Iranian isolates of *M. kansasii* are genetically diverse. Separation of isolates at high genetic distances in this study suggest the possible existence of undetected isolates that could fill the gaps between the unrelated isolates.

Keywords: MEE, *Mycobacterium kansasii,* Non-tuberculous Mycobacteria

Comparison of Oxacillin Agar Screening and PCR Methods in Detection of Methicillin Resistance *Staphylococcus epidermidis* Strains Isolated from Blood Cultures of Children

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Abstract

Background and Objectives: In recent years methicillin resistant *Staphylococcus epidermidis* is among the prevalent bacteria causing septicemia in neonates and children. Therefore the rapid diagnosis and their antibiotic resistance surveillance are of great importance for treatment. The aim of this study was to evaluate oxacillin screening agar with three concentrations of oxacillin and to compare with PCR in detection of methicillin resistant *Staphyliococcus epidermidis*.

Material and Methods: A total of 100 *Staphylococcus epidermidis* strains were recovered from blood cultures of neonates and children. Resistance of isolates to methicillin were checked using oxacillin agar screening containing 0.6, 4, and 6 µg/ml of oxacillin. They were also tested for *mec*A gene by PCR.

Results: According to PCR results 89 of test isolates revealed to have *mec*A gene. Oxacillin agar screening test containing 0.6 µg/ml oxacillin could detect 85 and 89 resistant strains after 24h and 48h incubation, respectively. The same test containing 4µg/ml oxacillin could detect 78 and 89 resistant strains after 24h and 48h incubation, respectively and by using 6 µg/ml oxacillin 67 and 75 resistant strains detected after 24h and 48h incubation, respectively.

Conclusion: Sensitivity of oxacillin agar screening test containing oxacillin concentrations of 0.6µg/ml and 4µg/ml after 48h incubation were the same as PCR test, whereas 6µg/ml oxacillin showed low sensitivity in detection of resistant isolates. Therefore, oxacillin agar screening test with 0.6 µg/ml and 4 µg/ml after 48h incubation, which is a cheap and handy for any level of routine laboratory could be used in accurate and effective detection of methicillin resistant *S. epidermidis* (MRSE).

Keywords: *Staphylococcus epidermidis*, Methicillin, Oxacillin screening agar, *mec*A gene

PCR detection of PER &VEB &SHV and TEM β-lactamases in multidrug resistant *P. aeruginoasa* isolated from wound infections in two hospitals of Tehran

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Abstract

Background and objectives: Class A serine beta-lactamases such as SHV, TEM, PER and VEB are among the most important resistance determinants emerging in gram negative bacterial pathogens. PER beta-lactamase is an enzyme of notable clinical importance due its ESBL (extended spectrum beta-lactamases) activity and fully characterized in *P. aeruginosa*. The present investigation was under taken to assess the prevalence of beta-lactamase genes belonging to SHV, TEM, PER and VEB in *P. aeruginosa* isolates from wound infections in two hospitals of Tehran.

Material and Methods: Totally 100 isolates of Multi Drug Resistant *P. aeroginosa* from wound infections were collected over one year period in two hospitals of Tehran. Susceptibility to antibiotics and MICs for ceftazidime and imipenem of these 100 isolates were assessed using disc diffusion and microdilution methods, respectively. Isolates showing MIC≥16 microgram/ml for ceftazidime were subjected to PCR using four primer sets including SHV, TEM, PER and VEB types of beta-lactamases.

Results: Only 6 of 100 studied isolates showed MIC≥4 for imipnem. Resistance to ceftazidime was determined in 42% of the isolates and 46% of the isolates showed MIC≥16 microgram/ml for ceftazidime. PCR assay indicated that out of 46 isolates showing MIC≥16 microgram/ml for ceftazidime, 28%, 11%, 13% and 11% were positive for SHV, TEM, PER and VEB beta-lactamase genes.

Conclusion: Imipenem was the most effective antibiotic against multidrug resistant *P. aeroginosa* isolates from wound infections in this research. SHV beta-lactamase was more observed than three other studied genes among the isolates recovered from wound in two studied hospitals of Tehran. It is the first report of *bla* $_{PER}$ and *bla* $_{VEB}$ in *P. aeruginosa* in Iran.

Keywords: SHV, TEM, PER, VEB, β-lactamase, *P. aeruginosa*

Rapid detection of *Pseudomonas aeruginosa* in clinical samples of burned patients by Fluorescent in situ hybridization(FISH)

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Abstract

Background and Objectives: *Pseudomonas aeruginosa* is one of the most important opportunistic pathogens in patients who suffer from immunodeficiency and burns. Wound infections caused by *P. aeruginosa* can rapidly change to systemic and life threatening situation. The aim of this study was to apply florescent in situ hybridization (FISH) method for rapid detection of *P. aeruginosa* in burrn patients.

Material and Methods: This study was performed on 100 samples of wound and blood from burn patients at Taleghani Hospital in Ahwaz. The samples were used for both bacterial culture and FISH assays. To use FISH technique, the samples were probed with the complementary sequences to specific region of 16 S rRNA. The probes were FITC- labeled pB-00375 specific for pseudomonas genus; CY3-labeled pb-00384 specific for *P. aeruginosa* and EUB-338 as a eubacterial probe. Due to labeling of the probes with fluorescent die, the fluorescent signals appeared shining after hybridization of the probes with the organism under the fluorescent microscope.

Results: Of 42 blood samples, 13 were positive for *Pseudomonas* in culture. Only one culture positive sample was recognized as negative by FISH. Sensivity and specificity of FISH for detection *P. aeruginosa* in blood samples was 94.7% and 100% respectively. FISH could detect all 20 culture positive samples from wounds corresponding to sensitivity and spicifity of 100% and 93/3% respectively.

Conclusion: When rapid diagnosis of infectious agents is required, FISH can be used as sensitive technique for detection of causative organism with a complete process of 3 hours. Moreover, due to visibility of morphology of infected organism in "FISH", recognition of infecting agent will be achieved confidently.

Keywords: FISH, *Pseudomonas aeruginosa*, Fluorescent, Probe

Prevalence of *Campylobacter jejuni* samples from patients referred to Semnan public health centers in 2007

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Abstract

Background and objectives: Diarrhea is endemic to developing countries. Different microorganism including *Campylobacter jejuni* can cause diarrhea. The prevalence of infection with this organism and its significance as causative of diarrhea is underestimated. The aim of this study was to determine the frequency of *Campylobacter jejuni* in Semnan hygiene centers diarrheic referrals.

Material and methods: stool samples (n=306) were collected by swabbing prior to antibiotic therapy of diarrheic patients. Samples were transferred to laboratory in Stuart medium. The swab was inoculated on Preston blood free campylobacter agar and incubated in 42ºC for 48 hrs. Suspected colonies were identified to species level using bacteriological tests. The Kirby-Bauer method was used to determine the susceptibility of isolates to different antimicrobial agents. .

Results: *Campylobacter jejuni* was isolated from 38 cases (12.4%). The most effective antibiotic was gentamycin (97.4%). Resistance to cotrimoxazole was the most (52.7%).

Conclusion: The prevalence of *Campylobacter jejuni* in this study is higher than other studies in Iran. *Campylobacter jejuni* should be considered as important causative of diarrhea in Semnan province.

Keywords: *Campylobacter jejuni*, diarrhea, health centers, stool, frequency

Molecular Detection of bacterial pathogens involved in urinary tract infection

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Abstract

Background and Objectives: Urinary tract infection (UTI) is one of the most common infections and a major cause of patient morbidity world wide. The gold standard for detection of bacterial pathogens in UTI is culture of urine specimens. In order to facilitate identification of bacterial pathogens in clinical urine specimens we designed a molecular method to detect bacterial pathogen in UTI.

Material and Methods: The genomic DNA of standard bacterial pathogens involved in UTI were extracted. Based on the 16S rDNA, the universal primers were designed and the amplification of the fragments was carried out. The enzymatic digestions were performed by two restriction enzymes (*Hae*III and *Alu*I) to produce a specific pattern for bacteria as follow; *E. coli*, *K. pneumoniae*, *S. saprophyticus*, *S. aureus*, *P. aeruginosa* and *P. mirabilis*.

Results: Although the size of the PCR products were not quite changed in different species, but the pattern of digestion is exclusive. The polyacrylamide gel electrophoresis showed a significant differentiations bands.

Conclusion: The digestion pattern can be used as a standard for identification of bacterial pathogens involved in UTI.

Keywords: UTI, Universal Primers, PCR, RFLP.

Assessment of the S-layer nanostructure production under anaerobic and 5% CO_2 conditions in *Lactobacillus acidophilus* ATCC4356

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Abstract

Background and Objectives: Surface layers (S-layers) are monomolecular crystalline arrays composed of protein or glycoprotein subunits. S-layers have been identified as the outermost structure of cell envelope in numerous organisms from the domains Bacteria and Archaea. The S-layer protein subunits are non-covalently linked to each other as well as to the supporting cell wall, and can be disintegrated into monomers by denaturing agents such as urea or guanidine hydrochloride. The attention to S-layers is due to their structural properties and the capability of self-assembly on various surfaces. These properties are the new fields of researches in the nanobiotechnology, nanotechnology, biotechnology and biomedical sciences.

Material and Methods: *Lactobacillus acidophilus* ATCC4356 was cultivated in MRS broth under anaerobic and 5% CO_2 conditions at 37°C. Surface proteins of *L. acidophilus* were extracted by treatment of whole cells with (4M) guanidine hydrochloride. Then these extracts were analyzed by SDS-PAGE.

Results: When the S-layer proteins were extracted and analyzed by SDS-PAGE, they were shown by coomassi brilliant blue staining. The S-proteins' bands of 43 kDa were visible and compared together.

Conclusion: It has been shown that the anaerobic condition is better than 5% CO_2 condition for extraction S-layer protein in *Lactobacillus acidophilus* ATCC4356.

Keywords: S-layer, Probiotic, Surface layers, *Lactobacillus acidophilus*

Comparison of the antibacterial activity of Handsept and Decosept

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Abstract

Background and Objectives: The etiology of nosocomial infections, the frequency of contaminated hands with the different nosocomial pathogens, and the role of health care workers' hands during outbreaks suggest that a hand hygiene preparation should at least have activity against bacteria, yeasts, and coated viruses. Hand washing is emphasized as the single most important measure to prevent cross transmission of micro-organisms and thus to prevent nosocomial infections. In this respect, we compared the antibacterial activity of handsept with decosept as two hygienic hand disinfections.

Material and Methods: In vitro activity of "Handsept" (Isopropanol, N-propanol, Benzalkonium chloride) and "Decosept" (Propan-2-o, Benzyl- C12- 16- alkyl dimethyl ammonium, propan-1-01) were tested against 4 reference standard strain and 6 clinical isolated. Samples including *Escherichia coli*, *Klebsiella spp*, *Staphylococcus aureus*, *Pseudomonas spp*. In vitro activity was established using broth dilution method, additionally, the in vivo anti-microbial properties of the two antiseptic agents were tested on the bacterial contaminated hands of volunteers.

Results: Our results showed that "Handsept" can prevent the growth of 10 bacterial strains after 15, 30, 40 seconds. In another study "Handsept" was applied to contaminated hands of the health care workers with (10^6 /ml) of bacterial suspensions, which after 15 seconds no bacterial was found on their hands.

Conclusion: The use of hygienic hand disinfection (e.g. Handsep and Decosept) in all these situations will be effective in preventing cross-transmission of nosocomial pathogens. Similar findings have been found for "Handsept" and "Decosept". Thus we suggest using "Handsept" instead of "Decosept" in hospitals given their efficacy is similar and "Hansept" produced in Iran and has a more reasonable pricing than "Decosept".

Keywords: Transient flora, Handsept, Decosept