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Application of Molecular Techniques for Detection of Parasitic Infections

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Abstract

Background and objective: In parasitology, routine laboratory methods such as optical microscope are usually used for morphological identification of parasites. Recent studies have focused on alternative methods to improve the diagnostic methods of parasites. These methods include serological and proteomics techniques using spectroscopy and molecular technologies. Molecular techniques are used for identification of parasites based on their structure and characteristics with high specificity and sensitivity. In this study, new molecular techniques for the diagnosis and identification of parasites are considered.

Materials and methods: We considered more than 400 articles with published dates of 1980 to 2017 from the available databases such as Science Direct, Google Scholar, PubMed, Iran Medex, Scopus, SID, Magiran and reference books. Finally, 92 articles were selected for further study.

Results: Current and new molecular methods such as PCR, RT-PCR, LAMP, Luminex xMAP, RAPD, AFLP, RFLP, NASBA, and microsatellites can be applied for the diagnosis of parasites infections with high sensitivity and specificity.

Conclusion: Molecular methods provide comprehensive assessments for the diagnosis, treatments and epidemiological studies of parasite infections and eventually control mortalities raised by parasites.

Keywords: *Parasitic infection, Diagnosis, Molecular techniques, Molecular epidemiology*

Resistance to Aminoglycosides among Methicillin Resistant *Staphylococcus aureus* Strains Isolated from Patients with Urinary Tract Infection in Isfahan, Iran. 2017

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Abstract

Background and objective: Aminoglycoside antibiotics are used to treat many Gram-negative and some Gram-positive infections. Among various bacterial species, resistance to AGs arises through a variety of intrinsic and acquired mechanisms, in which by far the most widespread mechanism of resistance to AGs is the inactivation of these antibiotics by AG-modifying enzymes. In this study, we aimed to determine the resistance to aminoglycosides among methicillin resistant *Staphylococcus aureus* strains in Isfahan.

Materials and methods: In this study a total of 286 *S. aureus* strains were isolated from patients with urinary infections in 2 hospitals in Isfahan during 2017 and identified using specific primers. The resistance of MRSA strains to 16 antibiotics was determined and presence of different SCCmec types and genes encoding resistance to aminoglycosides was detected.

Results: A total of 112 (39%) cefoxitin resistant and *mecA* positive *S. aureus* strains were identified among isolates. All MRSA strains were susceptible to vancomycin, linezolid and quinupristin/dalfopristin and showed high resistance to penicillin, ciprofloxacin, erythromycin and clindamycin. Moreover, 92, 89, 89, 86, 85 and 63% of strains were resistant to tobramycin, kanamycin, amikacin, neomycin and gentamicin, respectively. Ninety seven percent of strains harbored SCCmec type III and *aac(6')-Ie+aph(2')*, *ant(4')-Ia*, *ant(6)-Ia* and *aph(3')-IIIa* genes were detected among 63, 60, 56 and 38% of strains, respectively.

Conclusion: High prevalence of resistance to aminoglycosides among MRSA strains indicating the inefficiency of these antibiotics for treatment of urinary infections in this study.

Keywords: *Aminoglycosides, MRSA, UTI, SCCmec typing*

Zoonotic Parasites in Slaughtered Animals of Sanadaj Slaughterhouse

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Abstract

Background and objective: Zoonotic parasites are a broad range of diseases Which are of great health and economic importance. The purpose of this study was to identify the Zoonotic diseases in slaughtered animals in Sanadaj slaughterhouse.

Material and method: This cross sectional study was done on gastrointestinal tract 273 slaughtered animals including 164 sheep, 45 and 64 gastrointestinal tract of sheep, goats and cattle were collected, respectively, and transferred to the laboratory of Parasitology. Data analysis was done by SPSS software.

Results: Results showed that the prevalence of gastrointestinal parasites of sheep, goats and cattle were 29%, 38% and 8%, respectively. The prevalence of fasciola in the liver of sheep, goats and cattle were 5.49%, 4.4% and 25.6%, respectively. The prevalence of dicrocelium of liver: 3.05%, 2.2% and 3.1%, the prevalence of lung worms in sheep and goats were 8.54% and 4.4%, respectively, but there was no pulmonary infection in cattle. The prevalence of hydatid cyst in the liver of sheep, goats and cattle were 6.25%, 2.2%, 4.27%, the prevalence of hydatid cyst in lung were 7.32%, 11.11%, 9.37%, respectively. In this study, 19 parasite species from sheep (12 nematodes, 4 cestodes, 3 trematodes), 11 parasite species from goats (7 nematodes, 2 cestodes, 2 trematodes), 7 parasite species from cattle (3 nematodes, 1 cestode, 3 trematodes) were isolated.

Conclusion: According to our results, there is a high prevalence of Zoonotic parasites in Sanandaj livestock animals. Which, in addition to imposing economic losses and health risks for the human, Increases the need for wider health measures to control diseases

Keywords: *Zoonotic parasites, Alimentary tract, Lung, Liver, Slaughterhouse*

Antimicrobial Activity of *Taraxacum pseudocalocephalum* Leaves Extract on Pathogenic Microorganisms and Comparison with Common Therapeutic Antibiotics *in vitro*

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Abstract

Background and objectives: Dandelion with the scientific name *Taraxacum pseudocalocephalum* belongs to the Asteraceae family. The aim of this *in vitro* study was evaluated the effect of leaf extract of dandelion on *Candida albicans*, *Staphylococcus aureus*, *Bacillus cereus*, *Listeria innocua*, *Escherichia coli*, *Salmonella typhi* and *Pseudomonas aeruginosa* and comparing it's effect whit common antibiotics.

Materials and methods: In this research, leaf extract of dandelion was obtained by Maceration. Antimicrobial activity of leaf extract of dandelion was investigated using disk diffusion methods, well distribution in agar, minimum inhibitory concentration (MIC) (broth microdilution) and minimum bactericidal/fungicidal concentration (MBC or MFC).

Results: results of MIC of leaf extract of dandelion on *Candida albicans*, *Staphylococcus aureus*, *Bacillus cereus*, *Listeria innocua*, *Escherichia coli*, *Salmonella typhi* and *Pseudomonas aeruginosa* was 128, 128, 256,256,256,256 and 512 respectively. The most resistant strain to the aqueous leaf extract of the dandelion was the gram-negative bacteria of *Pseudomonas aeruginosa*. The result showed that the diameter of inhibitory growth zone in well diffusion agar method was more than disk agar diffusion and the strains showed bigger zone in lower concentration.

Conclusion: It can be stated that the aqueous leaf extract of dandelion on gram-positive bacteria and *candida albicans* showed more antimicrobial activity than gram negative strains, generally. According to the results of this study, further research on the antimicrobial compounds of the dandelion is suggested to be used in the treatment of infectious diseases.

Keywords: *Extract, Leaf of Dandelion, Pathogenic microorganisms, Antibiotic, Antimicrobial activity.*

Isolation of Vancomycin Resistant Enterococci from Hospital Sewage in Tehran. 2016

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Abstract

Background and objective: Members of the genus *Enterococcus* are well-documented pathogens associated with various clinical manifestations, including bacteremia, infective endocarditis, intra-abdominal infections, urinary tract infections, and, in rare cases, central nervous system infections. In this study, we aimed to isolate vancomycin resistant enterococci from hospital sewage in Tehran.

Materials and methods: During 2016, sampling was carried out from effluent of a hospital in Tehran. The sewage sample was diluted and filtered using saline and membranes were transferred onto m-Enterococcus agar plates supplemented with vancomycin and incubated at 37°C. All colonies were identified at the species level using specific primers and the resistance of different strains to vancomycin and teicoplanin was determined. The presence of 9 different vancomycin resistance genes was detected using PCR.

Results: A total of 92 enterococcus isolates consisting of *E. faecium* (80%), *E. faecalis* (17%) and *E. gallinarum* (3%) were collected. All strains showed resistance to vancomycin and teicoplanin discs and the minimum inhibitory concentration ranges of vancomycin varied from 2048 to 128 µg/ml and most of the isolates (82%) showed resistance to highest level of vancomycin. Three different *vanA*, *vanB* and *vanC* genes were detected and *vanA* gene was common among all vancomycin resistant strains. Moreover, the presence of *vanC* gene was limited to *E. gallinarum* strains.

Conclusion: The presence of vancomycin and teicoplanin resistant strains in the effluent of this hospital in Tehran indicating the inefficiency of sewage treatment system.

Keywords: *vancomycin, enterococcus, hospital sewage, teicoplanin*

Phenotypic and Genotypic Characterization of Methicillin and Erythromycin Resistance in *Staphylococcus aureus* Collected from Nasal Samples in Qazvin Medical Students

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Abstract

Background and objective: Macrolide, lincosamide and streptogramin B (MLS_B) are used in the treatment of staphylococcal infections. The excessive use of these antibiotics has led to the emergence of resistance in this organism. The present study aimed to access phenotypic and genotypic factors involved in resistance to methicillin and erythromycin in *S. aureus* isolated from nasal samples in students in hospital.

Materials and methods: This cross sectional study was conducted among 172 students in Qazvin hospitals in 2015. The bacterial isolates were identified by standard laboratory methods. Antibacterial susceptibility and D-test were performed using disk diffusion method. The presence of *ermA*, *ermB*, *ermC*, *msrA* and *mecA* genes was evaluated by polymerase chain reaction (PCR) method.

Results: Among 172 samples, 50 (29%) were *S. aureus*. In total, 4 (8%) isolates were resistant to erythromycin and clindamycin (cMLS_B phenotype), 12 (24%) isolates showed inducible resistance (iMLS_B), and 6 (12%) isolates were resistant to erythromycin and susceptible to clindamycin (MS phenotype). Twenty-four (48%), 10 (20%), 12 (24%), 4 (8%), and 6 (12%) isolates were positive for the presence of *ermA*, *ermB*, *ermC*, *msrA* and *mecA* genes, respectively.

Conclusion: The findings of this study showed the considerable rate of *S. aureus* isolates and genes conferring resistance against MLS_B antibiotics in nasal samples collected from students. Continuous monitoring and treating of nasal carriers of *S. aureus* is essential in medical centers.

Keywords: *Staphylococcus aureus*, Nasal carriage, Antibiotic resistance, D-test

Activity of Gemifloxacin against Levofloxacin – and Ciprofloxacin-Resistant *Escherichia coli* Displaying DNA gyrase Isolated from Patients Admitted to the intensive care unit

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Abstract

Background and objectives: Urethritis is a common infection in hospitals, especially in intensive care units (ICUs), whose treatment is difficult today due to increased prevalence of resistant bacteria. The aim of the present study was to determine the resistance pattern to gemifloxacin in fluoroquinolones-resistant *Escherichia coli* strains possessed DNA gyrase isolated from hospitalized patients in ICUs.

Material and methods: In this descriptive cross-sectional study, patients (n = 113) in ICUs were studied. After identifying of *E. coli* isolates using specific tests broth microdilution test was used in according to CLSI M100-S25(2015) criteria to determine the minimum inhibitory concentration (MIC) of ciprofloxacin, levofloxacin and gemifloxacin. The fluoroquinolone resistance gene (*gyrA*) was identified using polymerase chain reaction (PCR) with specific primers.

Results: In this study, the prevalence of *E. coli* isolates resistant to ciprofloxacin was reported 35.2%. Determining MIC of gemifloxacin showed that 87.3% of the strains were susceptible to it (MIC, $\leq 0.25 \mu\text{g/ml}$), while 53.5% MIC ($\leq 1 \mu\text{g/ml}$) and 46.5% o (MIC, $\leq 2 \mu\text{g/ml}$) of isolates were categorized as susceptible to ciprofloxacin and levofloxacin respectively. The concentration of gemifloxacin, that inhibited 90% growth of isolates (MIC₉₀) was $0.5 \mu\text{g/ml}$, 8-fold lower than levofloxacin (MIC₉₀= $4 \mu\text{g/ml}$) and 8-fold lower than ciprofloxacin (MIC₉₀= $4 \mu\text{g/ml}$). The study of the polymerase chain reaction on gemifloxacin-resistant isolates confirmed the mutation in serine 83 of *gyrA* gene.

Conclusion: We conclude that not only resistance to fluoroquinolones, but the frequency of *gyrA* gene in *E. coli* strains isolated from special hospitalized patients is increasing.

Keywords: *Escherichia coli*, Urethritis, Gemifloxacin, DNA gyrase

Identification of *Salmonella* and *E.coli* with *invA* and *lamB* Genes by Multiplex PCR in Chicken Samples

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Abstract

Background and objective: *Salmonella* and *Escherichia coli* are the most common food pathogens bacterial especially in chicken products. Rapidly, specifically and simultaneously detection of the pathogens by identification of special genes, is important. This study aim was identification of both *E.coli* and *Salmonella* in chicken products by using multiplex PCR with *lamB* and *invA* genes amplification.

Materials and methods: 100 chicken samples were obtained . Samples were washed by sterile saline water and were cultured in biochemical medium. *Salmonella* and *Escherichia coli* strains were purified and DNA extraction was performed. Then amplification of both *invA* and *lamB* genes were used by multiplex PCR.

Results: From 100 chicken samples, 9 samples and 26 samples were isolated by biochemical tests, *Salmonella* and *E.coli*, respectively. Multiplex PCR results showed frequency of *invA* gene was 9% and for *lamB* gene was 26% , As also, frequency of both genes in samples were 1%.

Conclusion: In this study, multiplex PCR results was based on *Salmonella* and *E. coli* special virulence genes was confirmed biochemical tests results. Therefore comparison of results showed Multiplex PCR for rapid and simultaneous detection of food pathogens has high sensitivity and can be a reliable method for controlling of food contamination.

Keywords: *Chicken products, E.coli, Salmonella, Special gene, Multiplex PCR*