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COMPARISON BETWEEN GENE CASSETTES ARRAY OF INTEGRON CLASS I IN MULTIDRUG RESISTANCE ACINETOBACTER BAUMANNII AND KLEBSIELLA PNEUMONIAE IN Iran

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Background: Integrons are considered to play a major role in multidrug resistance dissemination among gram-negative bacteria. These are genetic elements capable of integrating and mobilizing individual gene cassettes via a site-specific recombination mechanism.Antibiotic resistance in *Acinetobacter baumannii* and *Klebsiella penumoniae* is a major problem in the hospital and outbreaks caused by this organism have been reported frequently. The present study aimed at determining the prevalence of different classes of integrons and the characterization of integron class 1 gene cassettes in Iranian *A. baumannii* and *K. penumoniae* isolates.
Methods: A total of 63 multidrug resistance *A. baumannii* and 100 *K. penumoniae* isolates were collected from clinical specimens in the Vali-Asr hospital in the central province of Iran. The antimicrobial susceptibility for 15 antibiotics was determined by disk diffusion. The presence of different integron classes was investigated by PCR and the size of gene cassettes in class 1 integrons was then determined by PCR as well. Moreover, integron cassette arrays of isolates were delineated by RFLP and sequencing amplicons with different lengths.
Results: 90% of *K. pneumoniae* and 98.4% *A. baumannii* isolates carried class 1 integrons, whereas class 2 integrons were found in 2% and 15.9% of K.pneumoniae and *A. baumannii* isolates, respectively. None of the isolates harbored int3. The length of the amplicons ranged from 1000 bp to 3 kb and 500 bp to 3000 bp in *K. pneumoniae* and *A. baumannii* isolates, respectively. Sequencing of integrons of class 1 revealed the presence of many resistance genes such as aadA, aacA, aacC, dfrA, blaGES and blaIMP in *A. baumannii* and dfrA17,aadA5, aac(6')-Ib-cr in *K. pneumoniae*. Cassette combination aacC1-orfP-orfP- orfQ-aadA1 and dfrA17-aadA5 were most frequently found in *A. baumannii* and *K. pneumoniae*, respectively. We identified a completely new gene cassette, which contained aacA7-qacF-aadA5-blaIMP, this cassette has not been reported previously in *A. baumannii*.
Conclusion: Data from this study demonstrated that class 1 integrons are highly diverse and are associated with a variety of drug resistance phenotypes and drug resistance genes and Integron carriage was significantly associated with an increase in multi- resistance.

Keywords: Integron Class I, *Acinetobacter baumannii*, *K. pneumoniae*, Gene Cassettes

ISOLATION AND IDENTIFICATION OF BACILLUS SUBTILIS FROM THE SOIL SATURATED SALT LAKE QOM AND THE SAFETY AS FOOD PROBIOTICS

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Background: Health promoting microorganisms, probiotics, have been recently increasingly included in various food products and proposed for use as a food supplement or as a therapy for several infectious diseases. Probiotics based on *Bacillus* strains have been increasingly proposed for prophylactic and therapeutic use against several gastro-intestinal diseases. We studied the safety of one *Bacillus subtilis* that isolate from soil saturated salt lake Qom and after isolation and identification bacteria, we studied the safety of this *Bacillus* strain.
Methods: In this research, we assessed characteristics that are associated with safety of this *Bacillus* as a probiotic: Soil sampling was done first and then transferred to laboratory and phenotypic and genotypic tests were performed.After 16S rDNA sequencing, the safety studies were performed in bacteria for using as a probiotic.
Results: 16S rDNA revealed that this bacterium is *B. subtilis*. The strain tested was found to be sensitive to most of the antibiotics used.The strain was non-hemolytic, negative lecithinase and negative enterotoxin gene. There were no differences in the hematological indexes measured in the blood from control and treated mice. Persistence of spores in the mouse gastrointestinal tract counts spores was no longer within detectable levels after 12 days. The strain was shown to produce surfactin and biofilm. This isolate showed autoaggregation and had hydrophobicity in the xylene, chloroform and ethyl acetate. Spores were shown to be completely resistance to gastrointestinal tract (GIT) conditions and the strain produce antimicrobial activity using a panel of Gram-positive and negative bacteria.
Conclusion: The surfactin that produced could facilitate entry into cells by rupturing the phospholipid membrane. Biofilm-producing has been shown to remain bacteria in the GIT. Spores were shown to be completely resistance to exposure GIT. This is important as it ensures that the stated dose actually reaches the region of the GIT where it should exert its effect. Hydrophobicity and autoaggregation of the cell increases the level of adhesion and colonization on tissues. Data showed that the strain tested; there was no indication of pathogencity, infection or toxicity study in mice. An important aspect of safety assessments for potentialmicrobial food supplements is to ensure that no nterotoxins are produced by the bacterium and this bacteria didn'show that and no virulence factors like haemolysis and lecithinase are produced. In conclusion, as potential food supplements *B. subtilis* appeared to show safety.

Keywords: Toxicity, Safety, Probiotics, *Bacillus Subtilis*, Food

RISK FACTORS ASSOCIATED WITH COMMUNITY-ACQUIRED TEM PRODUCING KLEBSIELLA PNEUMONIA

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Background: Antibiotic resistance is one of the major challenges in bacterial infection control. ESBLs-(Extended spectrum beta lactamases) are the groups of enzymes in which enable the bacteria to become resistant to different types of antibiotics. The aim of this study is to investigate risk factors associated with TEM gene in the *Klebsiella pneumoniae* isolates which collected from the laboratories of the city.
Methods: The study was a case-control investigation. A case patient was defined as a patient who had one isolate of a TEM gene positive *Klebsiella pneumoniae* strain. A control patient was defined as a patient who had one isolate of a TEM gene negative *Klebsiella pneumoniae* strain. TEM gene was determined by using double disk diffusion antibiotic susceptibility technique and PCR Method.
Results: There were a significant correlation between history of antibiotic use over the past 3 month (OR = 3.68, 95% CI: 1.21–11.19 P<0.022), close contact with family working in hospital (OR = 7.29, 95% CI: 2.09–25.42 P<0.05) and home distance from hospital under 2 km (OR = 3.08, 95% CI: 1.10–8.58 P<0.032) with the presence of TEM Genes.
Conclusion: The main risk factors associated with community-acquired TEM producing *Klebsiella pneumonia* strains were history of antibiotic use over the past 3 month, close contact with family working in hospital and home distance from hospital under 2 km. More studies are needed to evaluate the role of these factors in order to control the spread of drug resistance.

Keywords: *Klebsiella pneumonia*, Risk Factor, TEM, Extended-Spectrum B- Lactamases

SOME MORPHOLOGIC AND MOLECULAR PROPERTIES OF NATIVE SINORHIZOBIUM MELILOTI BACTERIA FROM KHORASAN-RAZAVI

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Background: The aim of this study was to investigate about some morphologic and molecular properties of native Sino-*Rhizobium meliloti* bacteria from Khorasan-Razavi.
Methods: For this purpose, bacteria were isolated from the roots of alfalfa plants that were collected from some areas of Khorasan- Razavi and examined for some biochemical properties. Morphologic properties were evaluated using stereomicroscope and electromicroscope. The plasmid profile of isolates was studied by Chen method. Molecular identification carried out by polymerase chain reaction (PCR) using specific primers of 16Sr DNA (rd1: 5'-AGAGTTT-GATCCTGGCTCAG-3', fd1: 5'-AAGGAGGTGAC-CAGCC-3'). The PCR product of one sample was sequenced on both strands by using fD1and rD1 primers with an ABI 3730 XL automated DNA sequencer.
Results: All nine isolated bacteria were found to Gram-negative asporogenous rods and coccobasili. Their colonies were convex, mucoid and white to cream color and the diameter of colonies was sometimes different. Binary fission and peritrichous multiple flagella was some properties that observed by using electron microscopy. Plasmid pattern of isolates indicated two plasmids on agarose gel electrophoresis. The isolates showed a band with size 1500 bp in 1.5% gel agarose as the same reference strain using primers fd1 and rd1. DNA sequencing analysis showed similarities to the identified 16S rRNA gene from S. meliloti in the GenBank nucleotide database on the Internet at the National Center of Biotechnology Information (NCBI) website (http: //www.ncbi.nlm.nih.gov).
Conclusion: In present study, the properties of *S. meliloti* isolated bacteria were similar to other references and the nucleotide sequence of one sequenced isolate indicated to *S. meliloti* at the NCBI site.

Keywords: *SinoRhizobium meliloti*, N2 Fixation, PCR

A COMPARATIVE STUDY OF ANTIMICROBIAL ACTIVITY OF NISIN LOADED IN ALGINATE AND ALGINATE-RESISTANT STARCH MICROPARTICLES

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Background: This study was designed to compare antimicrobial activity of alginate and alginate-resistant starch microparticles against *Listeria monocytogenes*.
Methods: Nisin loaded microparticles were prepared by a w/o-emulsion external cross-linking procedure with three-weight ratio of nisin to alginate (4: 1, 2: 1 and 1: 1). For comparison of the antimicrobial activity of nisin encapsulated in alginate and alginate-resistant starch microparticles, the release of nisin from microparticles was studied in distilled water as release medium and activity of released nisin was determined using the food pathogen *Listeria monocytogenes* ATCC 19117in agar diffusion test. 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 37 ºC for 24 h. The diameter of inhibition zone was then measured. The amount of released nisin from microparticles was determined using bacteriocin standard curve.
Results: The nisin release from all formulations occurs in two stages: first, a rapid release phase, which corresponds to nisin being physically entrapped in the suface of microparticles, and second, the nisin being gradually released, until the nisin content was constant (66-76% in Alg microparticles and 61-70% in Alg-RS microparticles). The releasing of nisin depended on the initial bacteriocin content. When the nisin content increased, the amount of released nisin was found to decrease In addition, lower rate releases were found in Alginate-resistant starch microparticles. This lower rate release for Alginate-resistant microparticles in comparison to alginate microparticles was attributed to the presence of resistant starch in alginate matrix that retarded the release of nisin.
Conclusion: Cumulative release of nisin from Alginate-resistant starch microparticles occur in a controlled manner and antimicrobial activity of nisin in this microparticle is maintained for a longer period of time which this long lasting antimicrobial activity useful in food matrixes.

Keywords: Nisin, Alginate, Resistant Starch, *Listeria monocytogenes*, Antimicrobial Activity

DISTRIBUTION OF MAIN E. COLI PHYLOGROUPS ENCODING CTX-M ESBL IN FECAL ISOLATES OF POULTRY: A SURVEILLANCE STUDY IN FIVE POULTRY HOUSES IN TEHRAN

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Background: Association of avian-*E. coli* phylogroups with human diseases and role of these strains in dissemination of resistance genes to human ones are of major health concerns. This study was aimed to investigate phylogroup diversity and prevalence of ESBL encoding genes among the avian fecal *E. coli* isolates in five different poultry houses.
Methods: A total of 500 avian cloacal swab samples from five different poultry houses were collected over a six month period in 2013 in Tehran, Iran. Antimicrobial susceptibility testing was done against 13 antibiotics from 9 different classes according to the last CLSI guideline. ESBLs phenotypes were detected by using ceftazidime and ceftazidime-clavulanic acid disks. The presence and type of blatem, blashv, and blactx-M were determined by PCR and sequencing in these isolates. Diversity of phylogroups A, B1, B2 and D were analyzed among the isolates with confirmed ESBLs phenotype by PCR. The plasmid extracts were screened for carriage of β-lactamase genes.
Results: A total of 444 *E. coli* isolates were obtained from the studied samples (88%). Nearly 92.6% displayed MDR phenotype. A frequency of 5.4% (26) was confirmed for the isolates presenting ESBL phenotype, which blaCTX-M, bla TEM and blaSHV. were detected in 6, 10, and 5 strains, respectively. Coexistence of blaTEM and blaCTX-M (3 isolates) and blaTEM, blaCTX-M, and blaSHV (2 isolates) was also detected in these strains. The phylogrouping results showed prevalence of main phylogroups as follow: D (42.3%, 11), B1 (34.6%, 9), A (15.4%, 4), and B2 (7.7%, 2). Plasmid mediated transmission of the β-lactamase genes were found in 42% of the strains presenting ESBL phenotypes.
Conclusion: The colonization of the avian intestine with *E. coli* strains related to phylogroups D, as a source of extraintestinal pathogenic *E. coli* strains responsible for human diseases, and carriage of β-lactamase genes in these strains proposed them as a source of pathogenic strains in human food chain.

Keywords: *E. coli*, Multidrug Resistance, Phylogenetic Groups, Extended-Spectrum B-Lactamase, Poultry.

EVALUATION AND COMPARISION OF THE ANTIMIROBIAL EFFECT OF LACTOBACILLUS GASSERI ON SALMONELLA ENTERICA SEROTYPE ENTERTIDIS

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Background: *Lactobacillus* spp., as probiotic bacteria, are being increasingly studied for their inhibitory activity against pathogenic bacteria.there is some evidence that they are not effective against gram –negative bacteria.The objective of this study was to investigate the antagonistic activity of *Lactobacillus gasseri* (Lga C009) against resistant *Salmonella enterica* serotype Enteritidis (ATCC 13311).
Methods: Antimicrobial activity by using common bacteria causing gastroenteritis in children (*Salmonella enterica* serotype entertidis ATCC13311) were evaluated using three microbiological methods, Disk Diffusion Agar, Well Diffusion Agar and Agar spot test.
Results: The highest inhibitory effect achieved by Well Diffusion Agar with an inhibitory zone diameter of 0/2 ±0/7 mm and the minimum belonged to Agar spot test with an inhibitory zone diameter of 0/3 ±0/18 mm ($P < 0.05$). By comparison, above three methods, the well diffusion agar method was shown more sensitive than the other methods.
Conclusion: Using of *lactobacillus* bacteria in food can prevent of gastrointestinal infections and also, it can play a role of inhibitory effect on the common gastrointestinal microorganisms, particularly in elder person and children that is an important approach and strategic for human health.

Keywords: *Lactobacillus gasseri*, *Salmonella*, Antimicrobial Activity

PERSISTENCE OF MYCOBACTERIUM AVIUM PARATUBERCULOSIS IN LIGHVAN CHEESE AS TRACKED BY PROPIDIUM MONOAZIDE QPCR AND MGIT-MPN

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Background: The aime of this study was to investigate the behavior of Map in Lighvan cheese with special reference to strains of Map, inoculum level, and storage time.
Methods: One standard and two native strains of Map were inoculated (2 and 4 log cell/ml) to ewe's raw milk and were used for cheese making. Behavior of Map throughout the manufacture, ripening and storage of Lighvan cheese was tracked using propidium monoazide (PMA) quantitative real-time PCR (qPCR) and MGIT-MPN assay.
Results: PMA-qPCR and culture assay demonstrated comparable outcomes. Based on these results, inoculum level and storage time showed a significant effect on persistency of Map. Furthermore, during the storage period different behavior was observed among the various strains of Map.
Conclusion: Map could survive the 6 months of storage period and Lighvan cheese has potential to support the survival of Map.

Keywords: *Mycobacterium avium* paratuberculosis, *Propidium monoazide*, F57-Quantitative Real-Time PCR, MGIT-MPN

COMPARISON OF CAGE AND BABA VIRULENCE GENES EXPRESSION IN SPIRAL AND COCCOID FORMS OF HELICOBACTER PYLORI

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Background: Under stressful environment, a spiral form of *H. pylori* is transformed into a coccoid form. The coccoid formation is very important problem in recurrence of disease and antibiotic treatment failure in gastritis pateints. Our objective of this study was to determine the morphological transformation-process from spiral to a coccoid form under Amoxicillin stress and also to evaluate and comparison of the virulence genes (cagE and babA) expression in spiral and coccoid forms of *H. pylori*.
Methods: The standard ATCC 26695 *H. pylori* strain and 9 clinical isolates were used in this study. The existence of interest genes were detected by conventional PCR method. Minimal inhibitory concentration (MIC) of amoxicillin was determined using by E-test method. All of ten isolates were cultured under MIC, ½, ¼ and 1/8 MIC concentrations of amoxicillin. The induction of coccoid forms was examined periodically by gram staining and light microscopy. Then, the viability of induced coccoids was evaluated by flow cytometry method. The quantitative mRNA expression levels of cagE and babA genes were detected and compared in spiral and coccoid forms of *H. pylori* by real-time PCR method.
Results: The MIC and sub MIC (1/2) of amoxicillin could be induce the highest rate (100% and 98% respectively) of coccoids *H. pylori* after 144 h. Flow cytometry analysis revealed that the MIC and sub MIC (1/2 MIC) concentration of amoxicillin induced the viable coccoids (>80% and 4% respectively). Quantitative RT-real-time PCR showed that the expression of cagE and babA in coccoids were lower (1.9 and 6.1 fold) than spirals, however, the difference between cagE expression was not significant in two forms.
Conclusion: Amoxicillin in sub MIC concentrations is a potent stressor for inducing the viable but non-culturable (VBNC) coccoid form of *H. pylori*. Of interest was the expression of two important virulence genes (cagE and babA) in coccoids, however with lower rate than spirals. In conclusion, we have concluded that the coccoid cells may have a role inbacteria lresistance, persistency and disease recurrence.

Keywords: *Helicobacter pylori*, Gene Experssion, Structural Gene Virulence Gene, Spiral, Coccoid Form

THE SEROEPIDEMIOLOGY OF HELICOBACTER PYLORI INFECTION IN TABRIZ CITY

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Background: Because of the invasiveness of specimen collection for bacteriologic diagnosis and the expense of tests such as labeled urea breath tests, serology is the most feasible means of determining the population epidemiology of *H. pylori*. The aim of this study was to describe the seroepidemiology of *H. pylori* infection in Tabriz city.
Methods: *H. pylori*-specific ELISA for the presence of IgG antibodies was performed on a representative sample of 21429 (70.02% F, 29.98% M) sera from Tabriz in two years (March 2012 to March 2014), using validated serosurveillance methods. Serum samples were analyzed through qualitative and quantitative methods for anti-H.pylori IgG antibodies with a research use only available enzyme immunoassay kit, that is, "Anti-*H.pylori* IgG IBL ELISA". Anti-*H.pylori* IgG antibody levels greater than 12 U/ml was considered positive. Analysis was conducted using SPSS v15.0 and Epi info v3.2.2. The subjects were divided into nine groups according to age, Group I: Children under 10 years old, 1118 subjects, Group II: between 11-20 years old, 1756 subjects (1165 F, 591 M), Group III of between 21-30 years old, 4200 subjects (2976 F, 1224 M), Groups IV: 31-40 years old, 5072 subjects (3576 F, 1505 M), Group V: between 41-50 years old, 4299 subjects (3130 F, 1169 M), Group VI: between 51-60 years old, 3115 subjects (2307 F, 808 M), Groups VII: between 61-70 years old, 1252 subjects (861 F, 409 M), Group VIII: between 71-80 years old, 483 subjects (284 F, 199 M), Group IX: between 81-90 years old, 116 subjects (64 F, 52 M).
Results: The overall seroprevalence of *H. pylori* infection in Tabriz was 63.93% (63.77% F, 64.24% M), with no statistical difference between genders. Seropositivity rates increased progressively with age. In the first group, 23.61% the second group, 47.15%, the third group, 65.90%, the fourth group, 72.98%, the fifth group 62.59%, The sixth group, 69.92%, Group seven, 71.25%, Group eight, 60.45%, nine group, 62.93 %, The Anti *H. pylori* test was positive.
Conclusion: The prevalence of infection with *H. pylori* in Tabriz City was lower than rates reported in other developed countries, at 63.93%. This study provides important baseline measurements for future preventive measures including vaccine research and development.

Keywords: *Helicobacter pylori*, Seroepidemiology, ELISA

IN-SILICO INVESTIGATION OF MIR-222 IN H. PYLORI-ASSOCIATED GASTRIC CANCER

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Background: Little is known about the potential role of miRNAs in the carcinogenesis of gastric cancer induced by *H. pylori*. Bioinformatics databases were used to determine possible molecular functions and pathways of miR-222 as a proposed prognostic biomarker in gastric cancer.
Methods: MiR-222 targetome including validated and predicted targets were evaluated by miRTarBase and miRWalk databases. Expression of all miR-222 target genes was analyzed in both normal and tumor gastric cells using UniGene database. Finally, normal and tumor gastric expressing targetome of miR-222 were assessed in DAVID database for enrichment analysis of molecular pathways.
Results: DAVID database outcomes demonstrated that multiple KEGG signaling pathways including pathways in cancer, in gap junction and tight junction and in chemokine signaling pathway are the most engaged pathways induced by miR-222 targetome. Based on the majority of affected signaling pathways, miR-222 may results in progression and elevated invasiveness of gastric cancer by targeting MAPK signaling pathway.
Conclusion: miR-222 correlates with proliferation, apoptosis and cell division through tight junction and MAPK signaling pathways.

Keywords: Gastric Cancer, Microrna, *Helicobacter pylori*, Targetome, Signaling Pathway

A COMPARATIVE STUDY ON DIAGNOSTIC ASSAYS, PCR, MICROSCOPIC AGGLUTINATION TEST (MAT) AND CULTURE FOR DETECTION OF RODENT LEPTOSPIROSIS IN MAZANDARAN PROVINCE, Iran

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Background: Leptospirosis is caused by infection with *Leptospira* bacteria. The infection transfers from animals (commonly rats, cattle, pigs and dogs) to humans. Transmission occurs by direct or indirect contact with serum, urine or other biological fluids of infected animals. Thus, animals (especially rodents) can act as a source of infection for humans or other animals. It has a worldwide epidemic, but most in temperate and tropical regions especially in areas with rainfall and neutral and slightly alkaline soil such as northern Iran.
Methods: Rodents were trapped in 10 different areas of Mazandaran Province during the summer 2013, and 150 rodents were captured alive. Urine and kidney samples were collected from each rodent and then, samples were used for culture in accordance for isolation of live *Leptospira*. The presence of *Leptospira* DNA was evaluated in urine and kidney samples from all mice using nested PCR method. In sera samples, antibody titers and possible types of infecting serovarieties were tested by Microscopic Agglutination Test (MAT) using a panel of 20 strains of live *Leptospira* as antigens. The statistical analysis of the obtained data was done using SPSS version 19. A 'Pvalue'<0.05 was considered to be statistically significant.
Results: Live *Leptospira* were isolated from the kidney and urine samples of 3 mice (2%). In nested PCR method, DNA of *Leptospira* was found just in the one urine sample of rodent (0.7%) but it was detected from kidney samples of 16 rodents (10.7%). In addition, serological study was indicated, 21.2% of sera were positive (equal or/ higher than 1: 200) against one or more serovarieties. The dominant strain was *L. serjoehardjo* among 11 rodents (7.3%).
Conclusion: MAT is the better method than culture and molecular based methods (including nested-PCR) to detect the bacteria. In molecular based methods, using of kidney sample is better than urine sample and there is a higher probability for detection of bacteria. Culture method is not suitable for clinical diagnostic laboratory due to its time-consuming procedure despite the fact that living organism is isolated. This method only is used for research studies.

Keywords: *Leptospira* Culture, Leptospirosis, Microscopic Agglutination Test, Mat, Nested Pcr, Rodent

INTRANASAL IMMUNIZATION WITH RECOMBINANT FIMH FUSED WITH FLAGELLIN ENHANCES IMMUNITY AGAINST URINARY TRACT INFECTION

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Background: In this study, we evaluated the humoral immunity of recombinant fusion FimH.FliC of UPEC as a vaccine candidate against UTI by intranasal route.
Methods: The constructed FimH.FliC fusion protein was purified by Ni-NTA column and then LPS of the protein was removed by Triton x114. Mice were intranasally immunized three times with 25 μg of proteins (FimH, FliC or FimH.FliC proteins). Total IgG, IgG isotypes (IgG1, IgG2a), and IgA responses of immunized mice were measured by ELISA.
Results: All mice groups induced significant immune response against FimH and FliC as compared to control mice. Mice immunized with the fused FimH.FliC protein induced significantly higher humoral (Total IgG, IgG1, IgG2a and IgA) 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.
Conclusion: To date, there has been limited success in developing an efficacious vaccine against UTI. Among UPEC antigenic proteins, FimH was reported to be highly immunogenic and induces protective immunity against UTI in mice. Furthermore, we evaluated the adjuvant properties of FliC of UPEC strains in linking with FimH antigen. Our results indicated that the FliC as adjuvant increased FimH-specific IgG, IgG2a, IgG1 and IgA that shifted the immune responses toward a Th2 profile. Our results demonstrated the ability of the fusion protein in inducing the humoral responses.

Keywords: Urinary Tract Infection, Vaccine, Uropathogenic *Escherichia coli,* Fusion, Fimh, Flic

EPIDEMIOLOGICAL SITUATION OF BRUCELLOSIS IN THE PAST THREE DECADES IN Iran

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Background: Brucellosis is one of the most common infectious diseases in Iran, Middle East and the vast areas worldwide. As Brucellosis is transmitted from animals to humans (Zoonoses), in addition to debility of patients and complications from disease and also economic losses caused by the disease control strategies in human population, leads to a reduction in food resources due to reduced animal population because of abortion in livestock and economic burden inflicted on the population.
Methods: The required information has been gathering from the summary of patient information forms, which are sent monthly through provincial health centers. In Iran epidemiological care strategies done by probable case definition, monthly case reports and if necessary, sentinel surveillance of high-risk groups, and annual and current reports indicate the disease process during several years and its influencing factors affects the increasing or decreasing disease trend.
Results: Over the last three decades studying the disease since1982 till 2013, It can be seen that the cases since1989 till 2001 from 175 cases in hundred thousand people have been decreased to 25 cases in hundred thousand people.From 2001 till 2005 from 25 cases in hundred thousand people has increased to 39 cases in hundred thousand people. The disease process has been reduced from years of 2005 to 2010 so that from 39 cases in hundred thousand people decrease to about 16 cases in hundred thousand people and in recent years the disease has increased from 2010 until 2013. According to the latest information on the disease in 2013 cases of disease were 19086 of which incidence is 25 cases in hundred thousand people. Hamedan, Lorestan, Kurdistan, West Azerbaijan, Kermanshah had the highest pollution (incidence rate between 84-54 per 100,000). Maximum number of people is in the age group 15-24 years, the highest number of cases were in the spring and summer months and in rural areas (78%) more than the urban areas (22 %) and for both sex men are (61 percent) and females (39 percent).
Conclusion: Increasing vaccination coverage in veterinary livestock sector and widespread deployment of pasteurization factories and producing pasteurized milk and products during these years has been the most important factors in reducing the incidence of the disease.

Keywords: Brucellosis - Iran-Epidemiology

SEQUENCE VARIATION OF PERTUSSIS TOXIN S1 SUBUNIT GENE IN CLINICAL BORDETELLA PERTUSSIS STRAINS OF IranIAN ISOLATES

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Background: According to the importance of pertussis vaccine and the lack of fundamental studies about the polymorphism of virulence genes in Iran, the aim of the present search is investigation of s1 pertussis toxin gene polymorphism on circulating strains compared to vaccine strains.
Methods: 50 nasopharyngeal Dacron swabs from patients with a clinical diagnosis of pertussis sent to pertussis reference laboratory at the Pasteur Institute of Iran in 2008-2012. The cultivation, biochemical tests, the antiserum, and PCR were used to confirm *B. pertussis*. Sequencing of PCR products was performed to determine ptxS1 alleles. Two strains of BP 134 and BP 18323 as vaccine strains were studied.
Results: All 50 clinical isolates of *B. pertussis* were studied. All strains had the dominant allele ptxS1A. There was difference between the alleles of clinical strains and vaccine strains.
Conclusion: In recent years, a significant increase has been observed in the incidence of whooping cough in the world. Allelic changes in genes of virulence factors have been caused new strains resistant to both whole-cell and acellular vaccines. Although, whole-cell vaccine was introduced about 60 years ago and the vaccination program is administering to infants and children in Iran, we have observed a significant increase in the course of illness in recent years. Our finding about allelic shift of ptxS1 gene was similar to many European and American countries showing difference of dominant allele with vaccine strains.

Keywords: Polymorphism, Iranian, *Bordetella pertussis*, Pertussis Toxin,

THE WHITE LINE FORMATION, A REMARKABLE DEFENSIVE STRATEGY OF PSEUDOMONAS ON THE BATTLEFIELD

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Background: The purpose of our main researches has been to obtain genetic and functional characterization of WLIP lipopeptide and the interesting phenomenon of defensive white-line formation in *Pseudomonads*. This unique strategy of *Pseudomonas* could be useful in controlling clinical pathogens.
Methods: A mutagenesis approach combined with draft genomic sequencing (next generation sequencing) as well as in silico analyses was employed. In parallel, WLIP was extracted, purified and subjected to MS and NMR analyses for structural elucidation and confirmation.
Results: Although the structure of WLIP has been known for more than 2 decades, genetic analysis of its biosynthesis was not yet reported. We have characterized the WLIP-synthesizing NRPS system of *P. putida* RW10S2 and P. fluorescens LMG 5329. Both systems have several features in common, including three NRPSs genes located in two remote genomic regions, an export system and a LuxR-type regulator. In both species, similar phenotypes are associated with WLIP production: antagonism of some other bacteria, solid-surface motility, and biofilm formation. Remarkably, the *P. fluorescens* system (Wip) shows homology to viscosin-group NRPS systems, while the *P. putida* system (Wlp) is more related to systems that produce LPs belonging to other groups with structurally quite different CLPs, namely putisolvin and entolysin. This is an interesting case of convergent evolution.
Conclusion: We have characterized the WLR in P. putida and P. fluorescence for the first time. This is a remarkable defensive approach used by *Pseudomonas* species to battle their pathogenic target. Very recent published reports indicated that such reaction could be widespread than it was expected before.

Keywords: Drug Discovery, Non-Ribosomal Peptide Synthase, Lipopeptides

A RECOMBINANT SUBUNIT VACCINE BASED ON THE L7/L12 AND TOMP31 PROTEINS INDUCES PROTECTION AGAINST BRUCELLA INFECTION IN BALB/C MICE

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Background: Brucellosis is the most common bacterial zoonotic disease worldwide and no vaccine is available for the prevention of human brucellosis. In humans, brucellosis is mostly caused by *Brucella melitensis* and *Brucella abortus*. The Omp31 and L7/L12 are immunodominant and protective antigens conserved in human *Brucella* pathogens. The aim of this study was to evaluate the immunogenicity & protective efficacy of a recombinant protein vaccine encoding *Brucella* truncated Omp31 and L7/L12 proteins.
Methods: Bioinformatic tools were used to design the truncated Omp31 and L7/L12- TOmp31 fusion protein. The humoral/cellular immune response and protection levels against challenge with wild *B. melitensis* and *B. abortus* were evaluated in vaccine immunized mice and control groups.
Results: Vaccination of BALB/c mice with the recombinant fusion protein (rL7/L12-TOmp31) provided the significant protection level against both *B. melitenisis* and *B. abortus*. Moreover, rL7/L12-TOmp31 elicited a strong specific IgG response (higher IgG2a titers) and significant IFN-gamma/IL2 production and T-cell proliferation was also observed. The Th1 oriented response persisted for 12 weeks after final immunization.
Conclusion: rL7/L12-TOmp31 could be a new potential antigen candidate for the development of subunit vaccines against *B. melitensis* and *B. abortus*.

Keywords: *Brucella melitensis*, *Brucella abortus*, Truncated Omp31, L7/L12, Fusion,Recombinant Protein Vaccine

RECOMBINANT NLP PRODUCTION FROM STREPTOMYCES CYANEOFUSCATUS UTMC 2101IN E. COLI

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Background: Necrosis and ethylene inducing like family of proteins (NLPs) are microbial-derived herbicides, causing necrotic lesions in dicot hosts. Presence of nlp genes has been reported in fungi, oomycetes and bacteria including actinomycetes. In this study, we have over expressed nlp gene from *Streptomyces cyaneofuscatus* UTMC 2101 in *E. coli*.
Methods: Whole nlp gene from *Streptomyces cyaneofuscatus* UTMC 2101 (KF939123) was PCR amplified using a pair of primers which introduced EcoRI and HindIII restriction sites and ligated into identically cleaved pET26b as an expression vector. The final construct was transformed in *E. coli* BL21(DE3). PET26b-nlp plasmid was purified and sequenced. A recombinant colony of pET26b-nlp transformants was inoculated in LB broth with appropriate antibiotic. Final IPTG concentrations of 0.5, 1 and 1.5 mM and incubation temperature of 20, 25, 30 and 37 °C were tested to induce the highest recombinant protein production. The periplasmic fraction was collected by osmotic shock method. Both prepared fraction and cell-free culture medium were concentrated with trichloroacetic acid (TCA) precipitation. The periplasmic expression of the recombinant NLP was approved by SDS-PAGE. Non-recombinant pET26b transformant was used as negative control.
Results: Amplification of the genomic DNA of *Streptomyces cyaneofuscatus* UTMC 2101 with specific nlp primers, resulted in formation of a 828 bp long ORF, encoding for a putative protein with 275 amino acids. The deduced amino acid sequence was predicted to have molecular mass of 29.85 kDa and 90% identity with necrosis inducing factor from *Streptomyces flavissimus*. Investigating different IPTG concentration and incubation temperature revealed that 1mM IPTG at 20 °C is the optimum condition for soluble periplasmic nlp expression. No significant amount of recombinant protein was detected in TCA- precipitated cell-free culture medium. In biological assay of the periplasmic fraction, tobacco leaves necrosis was observed within 48 h after the first spray of non-concentrated periplasmic fraction of pET26b-nlp transformants, while the leaves remained intact during treatments with periplasmic fraction of the mentioned negative controls.
Conclusion: The obtained data of the current study provided valuable insights into developing a cost-effective bioherbicide to manage dicot weeds especially in monocots farmland using the crude periplasmic fraction of the recombinant *E. coli*.

Keywords: Periplasmic Expression, Bioherbicide, *Streptomyces cyaneofuscatus*

PREDICTION OF HA1 PROTEIN LINEAR EPITOPES IN INFLUENZA A VIRUS FOR MAKING VACCINATE

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Background: The present study was designed to in silico resolving the major obstacles in the control or in prevention of the diseases caused by avian influenza virus.
Methods: Bioinformatic analysis of influenza virus hemaglutenin large subunit (HA1) was done for specific epitope detection and bioinformatic tools BCPred, Pepito, Disco Tope, Ellipro were used for better understanding and characterizing the HA1 structure and selecting appropriate regions as effective virus epitops.
Results: Prediction of epitope sofewares output were identified protein of extracellular loops effective in surface protein. This result is following with results of experimental test on this protein.
Conclusion: Areas have been identified as a suitable vaccine for colonization and expression in the laboratory. Use of this recombinant vaccine is safe and the contrast of the complete protein lowly induced non especial antibody.

Keywords: Avian Influenza, Epitope, Hemaglutenin, Bioinformatic

BIOREMEDIATION OF NICKEL THROUGH METARHIZIUM SP. UTMC 5002

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Background: In this study, we isolated nickel resistance fungi and evaluated their potential for bioremediation application in acidic pH condition.
Methods: Soil samples were collected from heavy metal contaminated parts of Iran. Fungal colonies were isolated and purified on potato dextrose agar (PDA), pH 5.7, amended with 400 mg/l of nickel. The plates were incubated at 28 °C for 2 weeks. Purified isolates were tested for tolerance against 1000 mg/l of Ni in PDB, pH 2.5 with incubation at 28 °C for 7 days. Selected strains were screened for ability to grow in PDB containing 1000 mg/l of Ni, pH 1, 1.5 and 2. For molecular characterization of the final selected isolate, genomic DNA extraction and subsequently, PCR amplification using nu-ssu-1536 and nu-ssu-817 primers was performed. PCR product was sequenced by Macrogen. The sequence was compared with those of other validated species using BLAST program.
Results: Using Ni supplemented medium and cultivation conditions described before, a total of 120 fungal colonies were obtained from15 soil samples. Most of the isolates showed heavy growth on PDA plates. The entire isolates were preserved in University of Tehran Microorganisms Collection (UTMC). During the primary screening of the whole 120 purified isolates, 11 strains enabled to tolerate 1000 mg/l of Ni in acidic pH (2.5) in liquid culture.Among these 11 isolates, only 5 isolates grown in pH 2 (containing 1000 mg/l). No growth was seen in lower pH (1 and 1.5), with the same concentration of Ni. Finally, based on morphological characteristics and growth rate in presence of Ni, the strain UTMC 5002 was selected for phylogenic identification. Based on BLAST results, isolate UTMC 5002 belongs to Metarhizium anisopliae with 100% similarity. The nucleotide sequence data has been deposited at GenBank under accession numbers KM008603. This is the first report of Metarhizium sp. UTMC 5002 heavy metal removal and growth in acidic condition.
Conclusion: Isolation and characterization of acidophilic and acid tolerant fungi, which are capable to remove heavy metals from contaminated solutions, is a new field of interest in Bioremediation technology. Current data provide a prerequisite for developing an efficient fungal Ni biosorbent using, *Metarhizium* sp. UTMC 5002.

Keywords: Bioremediation, Biosorption, *Metarhizium anisopliae*, Nickel

OVER-PRODUCTION OF EPIDERMAL GROWTH FACTOR (EGF) IN E. COLI

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Background: Epidermal Growth Factor (EGF) is a monomer polypeptide, which is produce from different tissue in human. It has many applications in medicine and Pharmaceutical Science. The main source of EGF is Parotid gland. Production of EGF from animal's source has many disadvantages and it is not suitable for human applications. The best way for production of this protein is recombinant DNA technology, batch fermentation and High cell density culture for *E. coli*. In this study, we report the heterologus over-expression of EGF in *E. coli* BL21 (DE3).
Methods: *Escherichia coli* is the most widely used host for producing recombinant proteins. Using BL21 derived strain can reduce proteolyses of heterologus protein. In this study we increased several times recombinant human EGF production in *E. coli* BL21 (DE3) [pET28a-egf] by optimization media and temperature in induction duration. For this reason the transformed BL21 (DE3) with pET28a including synthetic gene kinetic cell growth and rhEGF production investigated at three different medium like LB, TB and 32Y with different temperatures such as 24, 28, 32 and 37°C. The results of experiments were analyzed by SDS-PAGE, and cell growth and recombinant protein production kinetics.
Results: The optimal expression of EGF in *E. coli* can be easily achieved when the growth conditions are properly controlled. Media components, induction time, growth temperature, and IPTG concentration have profound effects on the way in which recombinant protein is produced. In this study the maximum expression was obtained from TB medium at 28°C with IPTG concentration of 0.1mM. The last OD600 and dry cell weight at this condition was 12.2 and 5.61g/L respectively. The final yield was 32 % of total soluble proteins, which has an important value for EGF production in *Escherichia coli* .
Conclusion: Using rich medium like TB and decrease temperatures can improve the amount of protein considerably

Keywords: *Escherichia Coli*- Epidermal Growth Factor (EGF) - Over-Expression.

FREQUENCY OF MUTATIONS ASSOCIATED WITH RIFAMPICIN RESISTANCE IN MYCOBACTERIUM TUBERCULOSIS STRAINS ISOLATED FROM PATIENTS IN WEST OF Iran

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Background: Tuberculosis (TB) is a devastating infectious disease causing high mortality and morbidity worldwide. The most serious threat related to tuberculosis control is the recent emergence of drug-resistant tuberculosis strains. The aim of the present study was to identify various types of mutations in rpo B regionfrom rifampicin resistant strains isolated from sputum of tuberculosis patients.
Methods: Drug susceptibility testing of 125 *Mycobacterium tuberculosis* isolates was determined using the CDC standard conventional proportional method. Target DNA of *M. tuberculosis* was amplified by PCR, hybridized and scanned. We used the LCD arrayto detect mutations within the90 bprpoB region. Each array is a transparent, pre-structured polymer supports using a non-fluorescent detection principle based on the precipitation of a clearly visible dark substrate.
Results: Of the 125 *M.tuberculosis* isolates, 35(28%) were found to be rifampicin resistant that using the LCD array revealed point mutations at 9 different codons. as follows S512T(AGC→ACC)(17%) D516V (GAC→GTC) (20%) H 526D (CAC→GAC) (6%) H526R (CAC→CGC) (20%) H526Y (CAC→TAC) (23%) S531W (TCG→TGG) (8%).The most frequent site mutations were L511P (CCG→ CTG) (46 %) followed S531l (TCG→TTG) (40%) and D516Y (GAC →TAC) (26%).
Conclusion: Phenotypic testing is time-consuming and laboratory facilities. Therefore, there is a need for rapid molecular methods for detection of mutation in drug resistance. Microarray rpoB can be used to detect rifampicin resistance determining region (RRDR) associated site mutations of rifampicin resistant *M. tuberculosis* isolates.

Keywords: *Mycobacterium tuberculosis*; Rifampicin Resistance; Rpobgene

INCREASING ISOLATION RATE OF MOTT IN SOUTH OF TURKEY

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Background: Mycobacteria other than tuberculosis (MOTT) are environmental organisms capable of causing chronic disease in humans and cause increasingly serious infections especially in immunosuppressive patients. The genus *Mycobacterium* currently has more than 130 species, includes highly infectios *M. tuberculosis* complex, *M. Leprae* and new important threat *Mycobacterium avium* complex (MAC). However all of these cases, the diagnosis of MOTT is often difficult or unconvincing and it cause false diagnosis and treatments.
Methods: The aim of the present study was to describe the diversity of MOTT by sequencing and RFLP of hsp65 gene region and compare these two methods from clinical isolates received to Tropical Disease Center Region Tuberculosis laboratory in Cukurova which has strategical location with border to iraq, syria and Iran. We analyzed 2048 *Mycobacterium* species from 27.511 clinical materials of 9 different cities total of 19 dispansery and 2 region hospitals patients with between January 2012 – March 2014 in Cukurova region, Turkey. MOTT included in this study were isolated from clinical specimens of 49 patients had pulmonary infection, and 5 infections cases related to other sites (lymphonod, biopsy and abscess fluid). MGIT 960 system was used for the recovery of mycobacteria from clinical specimens and MPT64 card test was used for identification. Genetic characterization to species level was determined hsp65 sequencing and RFLP.
Results: Tweenty five patients presented NTM infections, of whom (23/25) manifested pulmonary symptoms, (3/25) presented lymphadenopathy and (5/25) represented cases of healthcare-associated infections. A total of 54 species were identified and included: *M. abscessus; M. bolletii; M. intracellulare M.alvei, M. porcinum, M. peregrinum* were the most frequent in pulmonary. Lymphadenopathy case was caused by M. fortuitum infection. We encountered *M. abscessus,* and *M. smegmatis* in cases of healthcareassociated infections. Our study is on going.
Conclusion: Our study showed the diversity of MOTT species in Cukurova region that associates pulmonary infections. And because of relationships and similar climate conditions Cukurova regions MOTT mapping will be represent closed country as Iran, Iraq and Syria.

Keywords: MOTT, RFLP, Hsp65

MECHANISM OF ANTIBACTERIAL ACTION OF SATUREJA KHUZISTANICA ESSENTIAL OIL

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Background: The essential oil of *Satureja khuzistanica* exhibits broad-spectrum antimicrobial activity. The major component of S. khuzistanica essential oil (EO) is shown to be carvacrol. We studied the mechanism of *S. khuzistanica* EO action on *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923.
Methods: Susceptibility of the two organisms to *S. khuzistanica* EO was measured by disc diffusion. Minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) were also determined using broth microdilution. To determine the mechanism of S. khuzistanica EO, overnight cultures of bacteria were subjected to MIC concentrations of EO for 1 h, External EO was removed by centrifugation at 8000 rpm and the cells were lysed, centrifuged and the supernatants were used to measure the internal EO concentration at OD range of 250-300 nm. The potassium ion concentrations of both bacteria were measured before and after exposure of the cell suspension to ½ MIC of the EO for 0, 30 and 60 minute by atomic absorption.
Results: The Disc test results showed marked susceptibility to the EO with inhibition zones of 26 mm for *E. coli* and 31 mm for S. aureus, MIC and MBC values confirmed the disc test results and were 0.62 mg/ml (0.08% v/v) for both isolates. UV spectrometry showed the presence of EO in the supernatants of EO treated *E. coli* and *S. aureus*, suggesting increased permeability of cell membranes. *S. khuzistanica* EO also induced leakage of potassium ions from *E. coli* and *S. aureus* cells at ½ MIC concentrations.
Conclusion: Inhibitory and sub-inhibitory concentrations of *Satureja khuzistanica* EO can affect membrane permeability and possible damage to the bacterial cytoplasmic membrane.

Keywords: *Satureja khuzistanica*, Essential Oil, Mechanism, *E. coli*, *S. Aureus*

UNIQUE BIODIVERSITY OF RADIATION RESISTANT BACTERIA ISOLATED FROM AN IranIAN RADIOACTIVE SITE AND ANALYSIS OF THEIR PIGMENTS

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Background: Radiation resistance has been detected dispersedly among bacteria and some microorganisms growing in natural radioactive environments. Herein, we provide data of isolation, molecular identification and pigments characterization of radiation resistant bacteria recovered from an Iranian radioactive site.
Methods: The soil samples were collected from uranium radioactive site and the physico-chemical properties of the soil samples were determined. Soil serial dilutions were cultured in Trypton-Glocose-Yeast extract agar and all distinct colonies irradiated by gamma and ultraviolet radiation at various doses using a 60Co source. The surviving bacteria were identified by morphology, biochemistry and 16S rRNA gene sequencing. In addition, bacterial pigments were analyzed by spectroscopic properties, HPLC and LC-MS systems and their antioxidant activity were assessed by DPPH activity.
Results: Among 30 pure colonies, five UV and gamma radiation resistant bacteria were isolated from a radioactive site. Phylogenetic analysis based on 16S rRNA gene sequencing reveal that the isolated strains were belonged to *Micrococcus lylae, Kocuria rhizophila, Rhodococcus erythropolis* and *Dermacoccus nishinomiyaensis*. In addition, these bacteria were coccoid in shape, Gram positive, catalase positive, mesophilic, non-motile, non-sporulating, aerobic and with yellow pigmentation.The results of HPLC and LC-MS analysis of pigments show that the bacterial pigments were belonged to novel astaxanthin. In addition, the most radio resistant bacterium was *Kocuria rhizophila* and it was resistant to gamma radiation (30 kGy) and UV light (400 j m-2).
Conclusion: The isolated strains are the first report on radio-resistant bacteria belonged to the different genus in Iran. We supposed that these isolates could be a candidate in industrial and bioremediation applications due to their strong antioxidant activities, pigment productions and radiation resistant properties.

Keywords: Radiation Resistant, Biodiversity, Phylogeny

POLYMORPHISM OF COA AND AROA GENES IN THE METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS STRAINS ISOLATED FROM NASAL CARRIAGE AMONG HOSPITALIZED PATIENTS IN WEST OF Iran

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Background: Methicillin resistant *Staphylococcus aureus* (MRSA) is an opportunist bacterial pathogen frequency isolated in the hospital and community environments. The main objective of this study was to determine genotyping of MRSA strains isolated from the noses of the hospitalized patients in the Kermanshah Hospital, western Iran by PCR-RFLP.
Methods: 1269 isolates from patients screened more than 48 hours of admission from different wards and 330 isolates from hemodialysis word of Kermanshah Hospital as the largest hospital in western Iran. *S.aureus* was identified by biochemical tests common methods and Api 20 staph test (Bio Merieux) and MRSA was identified by Oxacillin strip (Mast STOX).
Results: 258 *S. aureus* isolates were recovered from 1387 samples. 96 of that 82 isolates hospital-acquired and 14 isolates was community-acquired. Digestion of aroA gene amplified (1,153 bp) yielded only one distinctive RFLP pattern. All of the isolates were generated approximately 850 and 300 bp bands.
Conclusion: MRSA is an increasingly common cause of nosocomial infections. Our results are in agreement with those from others, who showed that a few specialized colons are responsible for the majority of MRSA nasal carriers. In this study, MRSA strains isolated in different wards of hospital were closely related when analyzed by coagulase gene typing.

Keywords: Methicillin-Resistant *S. Aureus*, Phenotypic, *Staphylococcus Aureus*, RFLP, Coa Gene, Aroa Gene

THE EVALUATION OF HEPATITIS G / C VIRUS IN AMONG HIV POSITIVE PATIENTS AND GBV-C/HGV AFFECT IN THE HIV DISEASE PROGRESSION

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Background: Individuals infected with the human immunodeficiency virus (HIV) are often co-infected with other pathogens, especially hepatitis viruses. HCV and GBV-C/HGV are blood-borne viruses that are highly prevalent among HIV/AIDS patients. The aims of this study were the evaluation of Hepatitis G Virus in among HIV Positive patients and GBV-C/HGV affect in the HIV disease progression.
Methods: One hundred and fifty HIV-infected patients (92 men and 58 women) were included in this study. Patients were categorized into four different high-risk groups: intravenous drug users (n=48), hemophiliacs (n=45), homosexuals (n=32) and heterosexuals (n=25). In addition, these patients are separated with history of kidney (n=35), liver (n=60) diseases and history of transfusion (n=55).RNA was extracted from 100 µl of serum or plasma using the RNX plus kit. Detection of GBV-C/HGV-RNA and HCV-RNA were performed using reverse transcription and nested PCR.
Results: GBV-C/HGV-RNA and HCV-RNA were found in 26.66% and 30% patients, respectively. The prevalence of GBV-C/HGV in intravenous drug users, hemophiliacs, homosexual and heterosexuals, history of kidney disease and history of liver disease group was 31.25%, 28.88 %,21.88%, 20%, 28.58% and 25% respectively.
Conclusion: The prevalence of GBV-C and HCV in HIV infected case varied in different groups. Our study shows a significant relationship between coinfection with GBV-C and HIV Virus, because after 4 years slows the progression of HIV disease and liver diseases in HIV-infected patients.

Keywords: Human Immunodeficiency Virus (HIV), GBV-C/HGV, HCV

CHARACTERIZATION OF OVERT AND OCCULT HEPATITIS B VIRUS INFECTION AMONG HTLV-1 POSITIVE HEALTHY CARRIERS IN THE NORTHEAST OF Iran; AN HTLV-I ENDEMIC AREA

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Background: To date, no studies have provided data on hepatitis B virus (HBV) prevalence among asymptomatic, healthy human T-lymphotropic virus (HTLV-I) positive carriers. This sero- and molecular epidemiology study was performed on patients in the Northeast of Iran, which is an endemic area for HTLv-I infection.
Methods: 109 sera were collected from HTLV-I positive healthy carriers who were admitted to Ghaem Hospital, Mashhad City. All were tested for HBV serology and subsequently, real time PCR was carried out on the samples, regardless of the results of the serology. Standard PCR and direct sequencing were applied on positive samples.
Results: All cases were negative for HBsAg. Anti-HBc and anti-HBs were positive in 34 (31.1%), and 35 (32%) individuals, respectively. There were 19 (17.4%) cases that were positive only for anti-HBs, and they had already received HBV vaccine, 16 (15%) were positive for both anti-HBs and anti-HBc, indicating a past-resolved HBV infection, 18 (16.5%) were isolated as anti-HBc, and 56 (51.34%) were negative for all HBV serological markers. Only one subject (0.9%) had detectable HBV DNA (2 153 copy/mL), and assigned as being an occult HBV infection.
Conclusion: The low prevalence of HBsAg, despite the high percentage of anti-HBc positive cases, might be related to the suppression effect of HTLV-I on surface protein expression. The low prevalence of HBV infection among HTLV-I positive healthy carriers from an endemic region indicates that the epidemiology of HTLV-I and HBV coinfection is related to the endemicity of HBV in that region, rather than HTLV-I endemicity.

Keywords: Htlv-I, Hbv, Coinfection

CHEMICAL COMPOSITION AND ANTIBACTERIAL ACTIVITY OF ESSENTIAL OIL FROM (ROSMARINUS OFFICINALIS) AND (MENTHA SPICATA) AGAINST ORAL PATHOGENS

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Background: The microorganisms evaluated in this study are considered risk factors for oral diseases such as caries and periodontitis; they can also reach the bloodstream triggering other diseases in the human body, like endocarditis, brain abscesses, throat infections, respiratory and gastrointestinal system infections and bacteremia. Thus, this study carried out for antibacterial activity of essential oil from *R. officinalis* and *M. spicata* against some oral pathogens; *Streptococcus mutans* and *Streptococcus* sobrinus.
Methods: The chemical composition of the essential oils from leaves *R. officinalis* and *M. spicata* was determined by gas chromatography (GC) and gas chromatography–mass spectrometry (GC-MS). Antibacterial activity of the essential oils of plants were determined in triplicate by using the microdilution broth method in 96-well.
Results: Carvon, Limonene and Menthone in leaf of *M. spicata* and 1, 8- cineol and alpha-pinene in leaf *R. officinalis* were the major components. The oils from *R. officinalis* and *M. spicata* leaves showed high antimicrobial activity, with minimum inhibitory concentrations 125 µg/ mL for the tested bacteria, including *S. mutans* and *S. sobrinus*. But for *M. spicata*, minimum inhibitory concentrations were determined 65 and 125 µg/ mL for *S.mutans* and *S. sobrinus*.
Conclusion: The essential oils of *R. officinalis* and *M. spicata* leaves have shown promising activity against oral pathogens. Our results indicated that some active components are present in oils, so this makes them particularly interesting for future studies and development of novel antimicrobial agents.

Keywords: *Rosmarinus officinalis, Mentha spicata*, Oral Pathogens

COMPARISON OF THE ANTIBACTERIAL EFFECTS OF THREE ENDODONTIC ROOT CANAL SEALERS (AH26, AH PLUS AND MTA FILLAPEX) ON ENTEROCOCCUS FAECALIS.

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Background: Successful endodontic treatment requires effective removal of the bacteria from the mechanically formed canal. Sealers used to fill the canal should also have antibacterial properties in order to prevent bacterial growth. In this study the antibacterial effect of three root canal sealers ; AH26, AH Plus and MTA Fillapex is compared using *E.faecalis* as the reference organism, which is one of the most resistant bacteria causing root canal treatment failure.
Methods: Five plates of Muller-Hinton agar were simultaneously inoculated with a standard solution of *E.faecalis* (ATCC 29212). Each plate was divided into 3 equal parts with a 6mm well in each section. Each well was used for one type of sealer. Freshly prepared sealers were then poured into their wells. The diameter of inhibition zone around each sealer was measured 24, 48 and 72 h after incubation at 37 O C. The entire experiment was repeated three times and the data was analyzed using ANOVA and Tukey with SPSS 19.
Results: Mean and SD of the diameter of the inhibition zones for AH26, AH plus and MTA Fillapex were 16.2, 1.014, 10.33, 0.617 and 6.67, 0.9 mm respectively. The observed difference was significant (P=0.013). There were no significant differences in three runs for each of the sealers.
Conclusion: AH26 had the highest antibacterial effect AH plus was also effective but MTA Fillapex had no antibacterial effect at all.

Keywords: Root Canal Sealers, Antibacterial Effect, *Enterococcus faecalis*.

MLVA TYPING OF ACINETOBACTER BAUMANNII AS A MULTIDRUG-RESISTANT ORGANISM, ISOLATED FROM TEHRAN HOSPITALS.

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Background: *Acinetobacter baumannii* has emerged as an important nosocomial pathogen, with ability to acquire antibiotic resistance genes. The aim of this study was to characterize the resistance mechanisms and genetic relatedness of MDR *Acinetobacter baumannii* isolates collected in Tehran hospitals.
Methods: The resistance phenotypes and genomic diversity of 123 *Acinetobacter baumannii* isolates obtained from 2 hospitals in Tehran from 2009 to2010 were determined using antimicrobial susceptibility testing and multilocus variable number tandem repeat (MLVA).
Results: Antibiogram analyses showed that the isolates were fully resistant to b-lactam antimicrobials and had high resistance rates to the other antimicrobial agents tested. 30 isolates (24.4%) were found to be MDR isolates. All MDR *A. baumannii* isolates were resistant to imipenem (MIC≥ 16 mg/L) and 29(96.7%) of them were resistant to Meropenem. 28isolates (93.3%) were positive for bla OXA-23-like, two were positive for bla OXA-24-like. All isolates possesed ISA-ba1in the upstream region of the OXA-23-like gene. MLVA typing of MDR isolates showed 7 clonal complexes and 16 singelton.
Conclusion: The population structure of *A. baumannii* isolates is diverse. Isolates possess seven resistance gene determinants that give rise to the MDR phenotype. These data provide a better understanding of *A. baumannii* epidemiology within the hospitals. Continuous surveillance is needed for monitoring the of MDR strains.

Keywords: *A. baumannii*, MDR, Gene Determinants,MLVA

FREQUENCY OF ADHESIVE VIRULENCE FACTORS IN CARBAPENEMASE-PRODUCING ACINETOBACTER BAUMANNII ISOLATED FROM CLINICAL SAMPLES IN WEST OF Iran

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Background: *Acinetobacter baumannii* is a significant opportunistic pathogen which causes severe infections related to catheters and ventilator. Adhesive virulence factors (VFs) are effective in *A. baumannii* adherence and pathogenicity. The aim of this study is to evaluate the frequency of adhesive virulence factors in carbapenemase-producing *A.baumannii.*
Methods: In total, 104 *A. baumannii* were collected from teaching hospitals of Kermanshah, Iran within March 2011 to March 2013. All the isolates were tested for antimicrobial susceptibility by Kirby-Bauer disk diffusion method. Carbapenemase-producing isolates were identified, DNA of isolates was extracted by boiling and it was investigated for the presence of adhesive virulence factors by PCR.
Results: Among 50 carbapenemase-producing isolates, the frequency of fimH and csgA genes was found to be 30(60%) and 27(54%), respectively. 20 isolates (40%) carried both of fimH and csgA genes, but 13(26%) carried none of these two genes. None of these isolates presented genes codifying for other different adhesive virulence factors include fimbriae Dr (afa/draBC), fimbriae S (sfa/focDE), fimbriae P (pap), capsule (kpsMT), fibronecting receptor (fnb).
Conclusion: Adhesive virulence factors are responsible for pathogenesis of bacteria. As adhesive VFs, fimbriae type I (fimH) and curli fiber (csgA) are participated in adherence and biofilm formation. These factors give bacteria the ability to be hidden from the host immune system, then they cause infections. More than 50% prevalence of fimH and csgA genes among 7 adhesive VFs studied in this research show that maybe there is a significant relationship between the presence of fimH and csgA genes and *A. baumannii* infections.

Keywords: *Acinetobacter baumannii*, Virulence Factors, Carbapenemase-Producing, Adhesive

MOLECULAR EPIDEMIOLOGY OF MBL-PRODUCING ACINETOBACTER BAUMANNII IN WESTERN Iran

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Background: Carbapenem-resistant *A. baumannii* has been isolated highly in recent years. Metallo-b-lactamases (MBLs) have been detected and reported from *A. baumannii* producing carbapenemases in recent times. The aim of this study was to determine both phenotypic and genotyping of MBL producing *A. baumannii* isolates.
Methods: A total of 110 *Acinetobacter* spp. were collected from clinical specimens of hospitals in Kermanshah,a western province of Iran. To screen the MBLs, E-test strips (AB Biodisk, Solna, Sweden) were used according to the manufacturer's instructions. DNA genomic of *A. baumannii* isolates was SmaI-digested and analyzed by CHEF Mapper PFGE
Results: 89MBL-producing isolates (80.9%) were found using E-test MBL. 68 out of 89 isolates were collected from ICU and 16 isolates from emergency and 5 isolates from children ward. Among 47 MBL producing *A.baumannii* selected for pulsed-field gel electrophoresis (PFGE) analysis, we obtained 7 pulsotypes including 4 common types and 3 single types.
Conclusion: Our study showed that most of the isolates of *A. baumannii* were obtained from ICU and most members of cloneA were collected from this ward. The presence of CloneA in ICU ward is warning.

Keywords: *Acinetobacter baumannii*, OXA-Type, PFGE

CLONAL EVOLUTION OF MULTI-DRUG RESISTANT ACINETOBACTER BAUMANNII BY PULSED-FIELD GEL ELECTROPHORESIS

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Background: The aim of this study was to determine the MDR isolates and the existence of OXAs genes among MDR isolates of *A. baumannii* collected from Kermanshah hospitals in west of Iran.
Methods: Fourty-two MDR *A. baumannii* isolates were collected from patients of Kermanshah hospitals. The isolates were identified by biochemical tests and API 20NE kit. The susceptibility to different antibiotics by disk diffusion method (CLSI) was determined. PCR was performed for detection of blaOXA-23-like, blaOXA-24-like, blaOXA-51-like and blaOXA-58-like betalactamase genes in isolates and clonal relatedness was done by PFGE (with the restriction enzyme ApaI). The patterns were then analyzed by Bionumeric software.
Results: This study showed high resistance to ciprofloxacin, piperacillin, ceftazidime and resistance to other antimicrobial agents and more spread of blaOXA-23-like gene (93%) in MDR isolates. Six clones were obtainedthrough PFGE method named in A (10), B (9), C (5), D (4), E (11) and F (3) that clone E was an outbreak and dominant in different wards of the hospitals.
Conclusion: An isolate from the emergency ward of the hospitals had indistinguishable isolates PFGE profile and similar resistance profile to isolates from intensive care unit (ICU) proposes that the transmission from ICU to emergency ward probably occursthrough patients or hospital staff contact.

Keywords: *Acinetobacter baumannii*, Multi-Drug Resistant, OXA-Type, Pulsed-field Gel Electrophoresis

ACINETOBACTER BAUMANII AND NON-BAUMANII IN Iran, EVALUATION OF ANTIBIOTIC RESISTANCE

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Background: *Acinetobacter* species have become increasingly resistant to antibiotics over the past several years and these days there is a significant challenge in treating these infections. They are important causes of nosocomial infections
Methods: In a prospective study, we evaluated 100 positive cultures of *Acinetobacter* from 100 patients in different wards of seven tertiary care hospitals in Tehran, Iran. PCR was used to determine the species of *Acinetobacter* E-test and Disk diffusion method were used to determine the resistance of isolated *Acinetobacter baumannii* and non-*baumannii*. Antimicrobial sensitivity to ceftazidime, cefepime, amikacin, imipenem, piperacillin-tazobactam, tigecycline and colistin was analyzed.
Results: In our study 89% of the isolated *Acinetobacter* was *baumannii* and 11% was non- *baumannii*. 70% of samples were isolated from male and 30% of the isolates from female patients. The most incriminated wards were intensive care and burn units. *Acinetobacter* was isolated from respiratory secretion in 38%, wound in 29%, tip of catheter in 14%, urine in 8%, blood in 4%, CSF in 4%, pleural fluid in 2% and brain abscess in 1% of samples. *Acinetobacter* was resistant to amikacin and ceftazidime in 100%, to cefepime in 94.5%, to piperacillin-tazobactam in 83% and to imipenem in 64% of all samples. Sensitivity to colistin was 100% and to tigecycline was 74.5% in our study.
Conclusion: *Acinetobacter* is an increasingly isolated pathogen. The prevalence of cephalosporins and carbapenem resistant *Acinetobacter* is high in our study. Colistin and tigecycline are the best choices for treatment of *Acinetobacter*

Keywords: *Acinetobacter*, Antibiotics, Resistant

IDENTIFICATION AND DRUG SENSITIVITY OF ACINETOBACTER BAUMANNII CLINICAL ISOLATES FROM AHVAZ AND TEHRAN

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Background: *Acinetobacter baumannii* is an emerging opportunistic nosocomial pathogen in hospitalized patients of intensive care units. The purpose of this study was to identify the *Acinetobacter* spp. clinical isolates to specie level, and determine their Multi Drug Resistance (MDR) and Extensively Drug Resistance (XDR) status.
Methods: 142 clinical isolates of *Acinetobacter* spp from laboratories of university teaching hospitals in Ahvaz and 55 clinical isolates of *Acinetobacter* spp from Tehran were investigated. All of the isolates were identified as species level using rpoB gene sequencing. Susceptibility testing using disk diffusion method was performed on *A. baumannii* isolates to determine MDR and XDR stratus. The results were interpreted according to the Clinical Laboratory Standards Institute (CLSI) guideline.
Results: Out of total 197 *Acinetobacter* spp., 131 were identified as *A. baumannii* based on rpoB gene sequencing. Susceptibility testing revealed that 109 (55%) of *A. baumannii* isolates were MDR and 22 (11%) were XDR. Drug resistance rate was higher among Ahvaz isolates in comparison to isolates from Tehran.
Conclusion: *Acinetobacter baumannii* is an emerging opportunistic nosocomial pathogen with a high prevalence of multiple drug resistance. rpoB gene sequencing is a useful marker to assign the isolates as species level in genus of *Acinetobacter*

Keywords: *Acinetobacter baumannii*, Rpob Sequencing, MDR, XDR.

STUDY OF EXPRESSION OF THE GENE ALPHA-6 IN MULTIDRUG-RESISTANT ACINETOBACTER BAUMANNII AGAINST THYME ESSENCE WITH REAL TIME PCR

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Background: The present study was aimed to investigate the inhibitory effects of Thyme essence on the expression of antibiotic resistance genes aphA-6 and Housekeeping DNA gyrase -A against Multidrug-resistant strains of *Acinetobacter baumannii* with Real time PCR technique.
Methods: Five Multidrug-resistant strains of *Acinetobacter baumannii* among seventy-five drug-resistant strains of *Acinetobacter baumannii* selected from hospitals in Tehran. AphA-6 and Housekeeping DNA Gyrase-A genes with PCR method were approved for studies. *Acinetobacter baumannii* ATCC #19606 was used in this study as a model reference strain. MIC values were determined by broth micro dilution assay recommended by the NCCLS. Thyme essence to the MIC was added to 1 ml Muller Hinton broth and after mixing, 1 ml of bacterial suspension (5 × 10 5 CFU / ml) was added to the medium, incubated at 37 ° C for 24 hours. After incubating bacteria, bacterial mRNA was extracted and transformed into cDNA and level of expression of aphA-6 and Housekeeping DNA gyrase-A genes in comparison to non-exposed Thyme essence was examined by Real time PCR.
Results: The major components of Thyme essence were thymol (28.8%) and carvacrol (23.46%). Thyme essence with MIC (0.45 l / ml) and an inhibitory effect on multidrug-resistant *A.bummanii* was found. The average zone of inhibition by Thyme essence on multidrug-resistant *A.bummanii* growth was 18.6 mm. Antibiotic susceptibility test results among seventy five *A.bummanii* strains areOxacillin (100%), amikacin (75%), kanamycin (68)%, gentamicin(60%),imipenem (60%) and (89%) were resistant to neomycin. Melting curve analysis showed the species- specific melting temperature patterns on 53°? differentiating *A.bummanii*. Thyme essence with MIC (0.45?l / ml) has the effect of reducing the expression of antibiotic resistance genes aphA-6 with Real time PCR method and no inhibitory effect on Housekeeping DNA Gyrase-A gene.
Conclusion: Thyme essence has strong inhibitory effects against *Acinetobacter* baumani. Therefore, due to the increasing resistance of pathogenic bacteria, Thyme essence can be used as a natural alternative for common antiseptic. Additional clinical researches are necessary to completely confirm the above results for practical purposes

Keywords: *Acinetobacter baumannii*, Thyme, Apha-6gene

STUDY OF INHIBITORY EFFECT OF ALCOHOLIC EXTRACT OF INNER STRATUM OF OAK FRUIT (JAFT) AND HYDRO ALCOHOLIC EXTRACT OF SUMMER BULB ON ACINETOBACTER IN VITRO

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Background: *Acinetobacter* is an important nasocomoial agent. Regarding the increase of resistant bacteria and identification of side effects of antibiotics, using plant drug with antibacterial effect could be appealing. This study aims to investigate the inhibitory effect of alcoholic extract of oak inner stratum and hydro alcoholic extract of summer bulb on *Acinetobacter* in vitro.
Methods: Oak inner stratum and summer bulb were collected and alcoholic and hydro alcoholic extractions were done. Inhibitory effect was carried out by disk diffusion and agar well diffusion method.
Results: Alcoholic extract of jaft had an inhibitory effect, but hydro alcoholic extract of summer bulb did not have any significant effect on this bacteria. The highest inhibitory effect of jaft was in 80µg/ml concentration.
Conclusion: Alcoholic extract of jaft has inhibitory effects on *Acinetobacter*, but hydro alcoholic extract of summer bulb does not have noticeable inhibitory effects.

Keywords: Alcoholic Extract, Summer Bulb, Jaft, *Acinetobacter*

ANTIMICROBIAL ACTIVITY OF ENDOPHYTIC BACTERIAL POPULATIONS ORIGINATED FROM MEDICAL PLANTS IN Iran

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Background: The aim of this study was the isolation, molecular identification and antimicrobial activity of endophytic bacteria isolated from medicinal plants in some areas of Iran.
Methods: From April to Jun 2013 and April to May 2014, 23 fresh plant samples were collected. The samples were washed in running tap water to remove the surface soils. After drying, the samples were subjected to five step surface sterilization procedure. The surface- sterilized tissues were aseptically crumbled into small fragments with a scalpel then cultured directly on the R3A, ISP2 and Blood agar plates. The plates were incubated at 28 ºC for 2 to 4 days. Each isolate was cultured in TSB medium and incubated at 28°C for 14 to 16 days then the antimicrobial compoundwas extracted through 3 methods. The extractions were tested against target bacteria. Method 1: just using a part of culture medium. Method 2: using hot. Method 3: using an ultrasonic device.
Results: From 23 medical plants, total of 23 bacteria were isolated. The isolates were identified by 16S rRNA gene sequencing as species level. The most abundant genera among the isolates were *Bacillus*. All isolates were used for extraction of their antimicrobial compound. The extractions obtained by method 2 and method 3 were tested against the target bacteria. Species belong to Actinomycets family made up the largest fraction of the isolates. Using method 1, just 2 of 23 isolates had an activity against the pathogenic microorganism. With method 2, sixty of 23 isolates (69%) exhibited activity against the tested pathogenic microorganisms. 43 isolates were active against *Bacillus* cereus and 26% were active against *Staphylococcus aureus*, 34% were active against *Bacillus subtilis* and 29.9% strongly active against *Klebsiella* pneumonia, 17% were active against *Citrobacter freundii*, 17% were active against Proteus mirabilis, 47/6% were active against *Shigella* and 17% were active against *Escherichia coli* . Thirteen out of 23 isolates (46%) exhibited activity against the tested pathogenic microorganisms with method 3. 16.6% were active against *Bacillus subtilis* and 34% were active against *Klebsiella pneumonia*. 34% were active against *Citrobacter freundii*. 34% were active against *Shigella*
Conclusion: 16S rRNA technique was useful to identify isolates as species level..

Keywords: Endophytic Bacterial, Plant, Iran

STUDY OF CLASS-D OXACILLINASE TYPES IN IMIPENEM-RESISTANT ACINETOBACTER BAUMANNII CLINICAL ISOLATES BY RAPD-PCR

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Background: The objective of this study was to investigate the genetic fingerprints of class D oxacillinase positive imipenem-resistant *Acinetobacter baumannii* clinical isolates.
Methods: Sixty clinical isolates of *A. baumannii* collected from Imam Hossein and Shahid Motahari Hospitals between October 2011 to April 2012 were employed. Presence of class D oxacillinases was shown by PCR and genotyping was performed using random amplified polymorphic DNA (RAPD-PCR). Correlation between class D gene carriage and RAPD-PCR patterns was studied among the isolates.
Results: Majority of the imipenem-resistant isolates carried class D oxacillinase genes of which, 80% carried OXA-23, 60% had OXA-51, 61.66% harbored OXA-58 and 20% of the isolates carried OXA-24. It should be noted that in most isolates there was more than one gene. RAPD-PCR revealed three clusters (A-C) on a similarity level of 70% among which 53.3% belonged to cluster A, 41.7% were placed in cluster B and 5% in cluster C. On a similarity level of 85%, 10 groups were observed (1-10) of which, 28.3% were placed in pattern 1, 18.3 % had 8 pattern. OXA-23 was observed in 80 % of the isolates among which 29.2% belonged to pattern1; 20.8% were in pattern 8 and 16.6% of them were in pattern 9.
Conclusion: OXA-23 was the most prevalent oxacillinase among our isolates. In addition, distribution of the majority of OXA-23 positive isolates in only 3 patterns suggests the clonal spread of these important pathogens.

Keywords: *Acinetobacter baumannii*, Imipenem-Resistant, Classd Oxacillinases, RAPD-PCR

EVALUATION OF ANTIBACTERIAL EFFECT OF ECHIUM AMOENUM FISCH. ET MEY.ON MULTIDRUG RESISTANCE ACINETOBACTER BAUMANNII ISOLATES FROM CLINICAL SAMPLES OF BURN WOUNDS

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Background: Borage (Echium amoenum Fisch. et Mey), is a wild member of Boraginaceae. This plant grows in different countries and also northern mountains of Iran. This medicinal plant has largely been used by Iranian folk as a mood enhancer, an anxiolytic, an inflammatory, laxative, emollients and also for treatment of infectious diseases. In this study the methanolic extract of dried flowers of Echium amoenom were tested on the A. baumanii isolates from clinical samples of burn wounds.
Methods: 30 multi-drug resistant *A. baumannii* strains, which were isolated from burn wounds at Motahari Hospital of Tehran, were selected. The plants were purchased from a famous Atari in Tehran in 2013 and the methanolic extract was prepared by Peculator apparatus. Antibacterial activity of the methanolic extract was evaluated by disc diffusion method based on CLSI protocol 2012.
Results: The mean of diameter of the inhibition zone for different concentrations of extract were; 9.967±6.139 mm at 4000 ppm, 13.37±5.45 mm at the 400 ppm, 13.53±5.49 mm at the 200 ppm, 14.77±5.17mm at the 100 ppm and 14.13±5.7806mm at the concentration of 50 ppm.
Conclusion: Clinical strains of the *A. baumannii* were almost highly resistant to imipenem which is the common choice of antibiotic therapy in hospitals. Due to the calculated p value ≤ 0.05 in this study, we can say that borage extract can be as good as or even better than the imipenem which is used in hospitals recently.

Keywords: *Acinetobacter baumannii*, Echium Amoenum Fisch

THE EVALUATION OF ANTIBACTERIAL EFFECT OF METHANOLIC EXTRACT OF CURCUMA LONGA AND ZINGIBER OFFICINALE AGAINST DRUG-RESISTANT ACINETOBACTER BAUMANNII ISOLATES FROM BURN INFECTION

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Background: Rapid multi drug resistant property of *A.baumannii* has become a great concern in clinical fields. Due to its resistance to almost all the commercial antibiotic classes in hospitals, searching for a new material has become an important matter. Hence, in this study the antibacterial effect of methanolic extract of Zingiber offinale and Crucuma lonaga has been evaluated against drug resistant *A. baumannii* isolates from burn infection.
Methods: 30 drug resistant *A.baumannii* strains, which were isolated from burn wounds at Motahari hospital of Tehran within 2013, were selected. The plants were purchased from a famous Atari of Tehran and the methanolic extracts of them were prepared by percolator apparatus. Antibacterial activity of the methanolic extract was evaluated by disc diffusion method based on CLSI protocol.
Results: The mean of diameter of the inhibition zone for different concentrations of extracts were; 12.30±6.03 mm at the 400 ppm, 11.47±5.40 mm at the 200 ppm,11.17± 5.03 mm at the 100 ppm, 10.52±4.70 mm at the 50 ppm and 10.46±4.02 mm at the concentration of 25 ppm.
Conclusion: Clinical strains of *A. baumannii* were almost highly resistant to imipenem which is the common choice of antibiotic therapy in the hospitals. This result can show the synergism of antibacterial property of the methanolic extract of C. longa and Z. officinale. Furtherin vivo studies are recommended.

Keywords: *Acinetobacter baumannii*, Curcuma Longa, Zingiber Officinale, Burn Wound Infection

MOLECULAR IDENTIFICATION OF ESBL GENES BLA -SHV, BLA-VEB, AND BLA-PER, IN ACINETOBACTER BAUMANNII ISOLATED FROM PATIENTS ADMITTED IN A UNIVERSITY HOSPITAL IN ISFAHAN

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Background: *Acinetobacter baumannii* has become one of the most important pathogens responsible for healthcare-associated infections and particularly affects patients admitted to intensive care units (ICUs). Prevalence of class extended-spectrum -lactamases (ESBLs) has been investigated in *Acinetobacter baumannii*. The aims of this study were to determine the prevalence of ESBL genes bla SHV, bla VEB, and bla PER in clinical isolates of *Acinetobacter baumannii* in a university hospital in Isfahan.
Methods: Within 8 mounths, 121 clinical isolates of *A. baumannii* were collected from Alzahra Hospital in Isfahan. To confirm the species of *baumannii* a PCR test for identification of blaoxa-51 genes was conducted. Antimicrobial susceptibility was done by testing resistance to cefotaxime, ceftriaxone, ampicillin-sulbactam, cefepime, meropenem,, tobramycin, amikacin, tetracycline, ciprofloxacin, trimethoprim sulfamethoxazole, Aztreonam by using the Kirby Bauer disk diffusion method. Finally all of isolates were evaluated by PCR for detection of bla-SHV, bla-PER and bla-VEB genes.
Results: Among 121 isolates in this study 44% were female and 56% were male. Samples were cultured from the Trachea (35%), urine (17%), and blood (10%). Most of the isolates (50%) were from ICU. Resistance rate to antibiotics was high: cefotaxime (100%), ceftriaxone (100%), ampicillin-sulbactam (33.9%), cefepime (99.2%), meropenem (100%), tobramycin (86.8%), amikacin (87.6%), tetracycline (92.6%), ciprofloxacin (100%), trimethoprim-sulfamethoxazole (99.2%), Aztreonam (100%). 62.8% isolates were XDR and 100% were MDR. Among all samples bla -SHV gene was detected in 16%, bla-VEB in 26.6% and bla-PER in 36.8% of isolates.
Conclusion: The result of this study shows the growing number of nosocomial infection associated with XDR *A. baumannii* complex leading to difficulties in antibiotic therapy. In this study Ampicillin-sulbactam with resistant rate of 33.9% was the most effective antibiotic. Also this study confirms the large dissemination of the gene bla-PER among *A. baumannii* in Alzahra Hospital and the results of antibiotic susceptibility revealed high rates of resistance to different groups of antibiotics.

Keywords: *Acinetobacter baumannii*, Drug Resistance, ESBL, Bla -SHV, Bla-VEB, And Bla-PER

ANTIBACTERIAL EFFECT OF SILVER NANO-PARTICLES ON ACINETOBACTER BAUMANNII

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Background: *Acinetobacter baumannii* is a Gram-negative, obligate anaerobic, non-fermented and non-motile bacterium. *Acinetobacter baumannii* has become a particularly important organism in the Intensive Care Unit (ICU). *Acinetobacter baumannii* plays a significant role in nosocomial infections. Multidrug-resistant *A. baumannii* is recognized to be among the most difficult antimicrobial-resistant Gram-negative bacilli to be controlled and treated. For this reason, we examined the efficacy of nanosilver material against different types of bacteria.
Methods: Silver nanoparticles with sizes of 20 nm were obtained from the Pishtazan nanotechnology (Mashhad-Iran). Then, silver nanoparticles serial dilutions (40000, 20000, 10000, 5000, 2500, 1250,625,312 ppm) were prepared in nutrient broth medium and 1.5 x 106 bacteria were added to each tube. After 24 h incubation at 37 °C, the MIC (minimum inhibitory concentration) and MBC (minimum bactericide concentration) were determinate by sub-culturing and colony counting on Mueller Hinton agar. Also, we evaluated the antibacterial properties of nano silver by disk diffusion and well diffusion methods (30 µl 10,000 ppm solution per well and per disk) in this research.This study antibacterial activity of silver nanoparticles was tested for 20 clinical *Acinetobacter baumannii* (collected from Imam Reza Hospital in Tabriz) as a standard strain.
Results: The MIC and MBC of silver nanoparticles (20 nm) were 1200 ppm and 2500 ppm for all clinical isolated *Acinetobacter baumannii*. The zone of dick diffusion and well diffusion methods respectively were 9 mm and 7 mm. The MIC and MBC results obtained for clinical isolates *Acinetobacter baumannii* showed no significant differenc.
Conclusion: *Acinetobacter baumannii* is susceptible to silver nanoparticles. Also the same MIC and MBC in multiple clinical strains suggest that there is no resistance to silver nanoparticles in *Acinetobacter baumannii*.

Keywords: *Acinetobacter baumannii*, Silver Nanoparticles, MIC And MBC, Well Diffusion

PREVALENCE OF BLANDM, BLAPER, BLAVEB, BLAIMP AND BLAVIM GENES AMONG ACINETOBACTER BAUMANNII ISOLATED FROM TWO HOSPITALS OF TEHRAN, Iran

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Background: The aim of this study was to determine the frequency of blaNDM, blaPER, bla VEB, blaIMP and blaVIM type genes among *A. baumannii* isolates from hospitalized patients in Milad and Loghman Hakim hospitals, Tehran-Iran from 2012 to 2013.
Methods: This study was conducted on 108 *A. baumannii* isolates collected from Milad and Loghman Hakim hospitals in Tehran, Iran. Antibiotic susceptibility tests were performed by Kirby-Bauer disc diffusion and Broth microdilution methods according to CLSI guidelines. The frequency of MBL (metallo-beta-lactamase) and ESBL (extended-spectrum-beta-lactamase) producers were evaluated by CDDT (Combined disk diffusion test). The blaNDM, blaPER, blaVEB, blaIMP and blaVIM genes were detected by PCR and sequencing methods.
Results: The resistance of *A. baumannii* isolates tothe tested antibiotics were as follow: 103 (95.4%) to ceftazidime, 108 (100%) to cefotaxime, 105 (95.7%) to cefepime, 99 (91.7%) to imipenem, 99 (91.7%) to meropenem, 87 (80.6%) to amikacin, 105 (97.2%) to piperacillin, 100 (92.6%) to ciprofloxacin, 103 (95.4%) to piperacillin/tazobactam, 44 (40.7%) to gentamicin, 106 (98.1%) to ampicillin/sulbactam, 106 (98.1%) to co-trimoxazole, 87 (80.6%) to tetracycline and 1 (1.8%) to colistin. Using combined disk diffusion test, it was found that out of 108 cefotaxime-non-susceptible *A. baumannii* strains, 91 (84.2%) were ESBL producers and out of 99 imipenem non-susceptible *A. baumannii* strains, 86 (86.86%) were MBL producers.The prevalence of blaPER-1 and blaVEB-1 genes among 91 of ESBL-producing *A. baumannii* isolates were 71(78.03%) and 36(39.5%), respectively. The prevalence of IMP-1 and VIM-1 genes among metallo-beta-lactamase-producing *A. baumannii* isolates was 3 of 86 (3.48%) and 15 of 86 (17.44%) respectively and also confirmed for blaOXA-51 gene by PCR. Fortunately, blaNDM gene was not detected in isolates.
Conclusion: The prevalence of ESBLs and MBLs-producing *A. baumannii* strains is a major concern and highlights the need of infection control measures including prompt identification of beta-lactamase-producing isolates and antibacterial management.

Keywords: *Acinetobacter baumannii*; Extended-Spectrum-Beta-Lactamases (Esbls); Metallo-Beta-Lactamases (Mbls)

EVALUATION OF THE PATTERN OF ANTIBIOTIC RESISTANCE AND INCIDENCE OF BROAD-SPECTRUME BETALACTAMASES TYPE VEB AND INTEGRON CLASS1 AT ACINETOBACTER ISOLATIONS SEPARATED FROM CLINICAL SPECIMEN AT EDUCATIONA

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Background: *Acinetobacter* has emerged as a significant opportunistic pathogen responsible for nosocomial infections. Treatment of infections due to this organism is becoming a serious clinical concern and these bacteria are frequently resistant to multiple classes of antibiotics such as Family of beta-lactam drugs. The b-lactamase enzymes represent the main mechanism of bacterial resistance to b-lactam antibiotics. This study was conducted to determine the prevalence of VEB and INT 1 in *Acinetobacter* isolates from sari hospital
Methods: The study included 100 *Acinetobacter* isolates that were isolated from various clinical specimens. Susceptibility of isolates to the antibiotics was determined by standard disk diffusion method.ESBL production was determined by combination disk method. Using disks containing ceftazidim and cefotaxim alone and incombination with Clavulanic acid and VEB and Integron class1 producing genes wasdetected by PCR test.
Results: Among all *Acinetobacter* isolates, the highest resistance was observed for cefotaxime (100%), ceftazidim (100%), ceftraiaxone (96%), whereas the highest susceptibility was observed forcolistin (65%) Gentamycin (37%),tobramaycin (27%). Combined Disc Test showed that 24% of isolated were ESBL positive and among them 16.6% and 75% were positive for blaVEB and INT1 genes.
Conclusion: According to the results, most of the isolates of acintobacter are drug resistant. The number of isolates of β lactamas producer is 24% of the total samples. Thus, other mechanisms such as secretory pump and purines can have a role in drug resistance.

Keywords: *Acinetobacter*, Esbl, Antimicrobial Sesistant, Blaveb, Integron Class 1

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BIOFILM FORMATION AND ANTIBIOTIC RESISTANCE OF ACINETOBACTER SPP. ISOLATED FROM SKIN AND WOUND INFECTIONS

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Background: Some bacteria cause severe infections in compromised patients and they survive on abiotic surfaces in hospital environments and colonize on different medical devices. On this basis, samples of skin and wound infections were collected from three hospitals in Kerman, Iran
Methods: All isolates were characterized with conventional biochemical methods. Antibiotic susceptibility of all isolates was carried out with nineteen antibiotics from different groups by disc diffusion technique. Minimum inhibitory concentration (MIC) of seven antibiotics was determined against all isolates by agar dilution method (1-1024) µg/ml. The cell surface hydrophobicity (CSH) of all isolates was determined and four isolates with the highest CSH were evaluated for biofilm formation on different surfaces such as glass, polycarbonate, polypropylene and venous catheters.
Results: Twenty three *Acinetobacter* spp. were identified. According to Antibiotic susceptibility tests, all *Acinetobacter* strains were resistant to Cephalosporins and Quinolons (95.6%), Aminoglycosides (91.3%), Sulfanamids (95.6%), Tetracyclin (47.8%), Carbapenems (100%) and Colistin (8.6%). MIC of seven antibiotics except Colistin against all isolates was more than 128 µg/ml. Biofilm formation of the four selected isolates on glass and polypropylene tubes indicated denser aggregates on polypropylene than glass surfaces. The number of bacteria that adhered to venous catheter surface was reduced after treatment of culture with Colistin.
Conclusion: One of the isolates with highest biofilm formation was identified by 16S rRNA technique as *Acinetobacter baumannii* Iliya and registered as a new strain, which is a nosocomial agent, and its high colonization activity on medical devices was precisely proved in this research.

Keywords: Wound, Antibiotic Sensitivity, Hydrophobicity, Biofilm Formation

INVESTIGATION OF TIGECYCLINE RESISTANCE AMONG METALLO-BETA-LACTAMASE PRODUCING ACINETOBACTER BAUMANNII ISOLATES IN AN EDUCATIONAL HOSPITAL OF ESFAHAN

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Background: This study was designed to investigate the tigecycline resistance among metallo-beta-lactamase producing *Acinetobacter baumannii* isolates in an educational hospital of Esfahan.
Methods: In this cross-sectional study, 107 *A. baumannii* recovered from trachea, bronchial, wound urine; were identified by standard biochemical tests; and conformed as *Acinetobacter baumannii* species using blaOXA-51- like gene and PCR method. Antimicrobial susceptibility to 15 antibiotics was performed using Kirby-Bauer disc diffusion method; also, strains of *A. baumannii* non-susceptible to imipenem were screened for MBL production. Determination of minimum inhibitory concentration to colistin sulfate was carried out using E-test method. Combination disk was used for detection of MBL production. A solution was prepared by dissolving 186.1 g of disodium EDTA.2H2O in 1,000 mL of distilled water and adjusting it to PH 8.0. Two 10 µg imipenem disks were placed on the plate, and 10 µl of a 0.5 M EDTA solution was added to one of them. Increasing of inhibition zone with imipenem-EDTA ≥ 5 mm was determined as MBL producing isolate. According to EUCAST interpretative criteria, diameter of inhibition zone ≥18 mm sensitive and 15> mm resistant were considered.
Results: Totally, 100 isolates (93.45%) were MBL producers. Most of the isolates were obtained from the intensive care unit (68%) and the isolates were obtained from different clinical specimen; which were obtained from trachea (40%), bronchial (14%), wound (12%), urine (11%) and others (23%). Resistance rates were 99%, 96%, 85%, 79% and 56% against ceftazidime, imipenem, gentamicin, trimethoprim/sulfamethooxazole and amikacin respectively. The results revealed that 70% of our isolates were strongly sensitive to tigecycline and 26% were intermediately sensitive. No isolate was resistant to colistin sulfate.
Conclusion: The antibiotic resistance to most of the antibiotics is very high and resistance against tigecycline is increasing which can be caused by over expression of efflux pumps. In addition, colistin sulfate was the most effective antibiotic to be used in *A. baumannii* infections.

Keywords: *Acinetobacter baumannii*; Metallo-Beta-Lactamases; Tigecycline

PREVALENCE OF ESBL AND MBL IN ACINETOBACTER BAUMANII ISOLATED FROM IranIAN PATIENTS DURING THE RECENT 10 YEARS

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Background: Beta-lactam resistance appears to be primarily caused by β-lactamase production, including the extended spectrum β-lactamases, AmpC beta-lactamases, metallo-β-lactamases and oxacillinases. They are mostly consisting of KPC, VIM, IMP, NDM, and OXA-48 types. The aim of this study was to review the data about prevalence of ESBL and MBL in *A. baumanii* isolated from Iranian patients that were published during the recent 10 years.
Methods: The scientific search and data collection were done by two researchers. *Acinetobacter*, MBL, ESBL and antibiotic resistance in Persian and English were used as keywords. Scientific database including google scholar, pubmed, and all medical scientific journals and also abstracts of the Microbiology Congress were searched. All articles related to detection of MBL and ESBL using phenotypic methods (E-test, Double Disk Synergy Test, Combined Disk, Hodge test) and genotypic (PCR) were collected. The data including the method of detection, number of isolates, number of ESBL and MBL and their genotypes and …, were extracted.
Results: Among 48 selected articles, 20 articles were met the inclusion criteria finally. In total 1857 isolates have been subjected to ESBL and MBL phenotypic and genotypic analysis in different cities. The prevalence of ESBL among 694 isolates that were analyzed for that was 42.2% (293/694). The prevalence of MBL among 366 samples that were tested for that was 68.6% (251/366). The most common genotypes for MBL and ESBL were IMP-1 (2.06%) and PER-1) 47.5%) respectively. Detail of prevalence of MBL and ESBL genotype will be shown in a table in poster.
Conclusion: In order to monitor and control the spread of horizontal transfer of resistance; genotyping studies are essential.

Keywords: *Acinetobacter*, MBL, ESBL, Antibiotic Resistance

ANTIMICROBIAL ACTIVITY OF LAVANDULA ANGUSTIFOLIA MILL. ESSENTIAL OIL

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Background: *Lavandula* genus, the member of Lamiaceae family comprises thirty known species among which *Lavandula angustifolia* is economically important in toiletries and perfumery industries. *L. angustifolia* is commonly known in Iran as "Ostokhoddous" and is rich of essential oil. *L. angustifolia* oil has several medicinal and biological properties such as antispasmodic, carminative, diuretic, sedative, anti-inflammatory and analgesic, anthelmintic agent.
Methods: The aim of this research was to evaluate the chemical composition of *L. angustifolia* oil by GC and GC-MS and its antimicrobial activity by disc diffussion and microbroth dilutution in vitro condition.
Results: GC and GC-MS analysis of essential oil showed the presence of 1,8-cineole (34.8%), borneol (24.6%), camphor (10.4%) as the main components. The antimicrobial evaluation by disc diffusion and microbroth dilution against *S. aureus, S. saprophyticus, B. cereus, E. coli, S. typhimurium, C. albicans, A. niger* exhibited that *B. cereus* (IZ=18.4 mm, MIC and MLC= 2,4 µl/ml), *S. aureus* (IZ=14.9 mm, MIC and MLC= 2,2 µl/ml), *S. saprophyticus* (IZ=13.0 mm, MIC and MLC= 2,4 µl/ml) were susceptible microorganisms, while *C. albicans* (IZ=10.5 mm, MIC and MLC= 8,16 µl/ml), gram negative bacteria (IZ=7.0-8.0 mm, MIC and MLC= 2-8; 4-8 µl/ml) and *A. niger* (IZ=7.0 mm, MIC and MLC= 16,32 µl/ml) were less sensitive microorganisms than gram positive bacteria.
Conclusion: From this explanation, the present antimicrobial activity of *L. angustifolia* oil against gram positive bacteria is related to its main components and because of lower antimicrobial of this component compared to phenolic compounds such as thymol or carvacrol, *L. angustifolia* oil appears as a medium antimicrobial agent.

Keywords: *Lavandula angustifolia*, 1,8-Cineole, Borneol, Camphor, Gram Positive Bacteria

PREPARATION AND IN VITRO EVALUATION OF ANTITUMOR ACTIVITY OF TGFAL3-SEB AS A LIGAND-TARGETED SUPERANTIGEN

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Background: In this study, we explored the antitumor potency of tumor-targeted super antigens (TTS) strategy; we designed and produced a fusion protein as a new antitumor candidate by fusing the third loop (L3) of transforming growth factor α (TGFαL3) genetically to staphylococcal enterotoxin type B and evaluated in vitro antitumor activities against murine breast cancer cell line.
Methods: We designed and prepared TGFαL3-SEB chimeric protein and evaluated superantigenic activity, binding property to cancer cell over expressing epidermal growth factor receptor (EGFR) and in vitro antitumor activities.
Results: Cloning of tgfαl3-seb was confirmed by colony-PCR, enzymatic digestion and sequencing. The recombinant TGFαL3-SEB fusion protein with molecular weights of 31 kDa was expressed and confirmed by anti-his western-blot analysis. TGFαL3-SEB fusion protein attached to A431 cell line with proper affinity and induced dose-dependent cytotoxicity against cancer cells expressing EGFR in vitro.
Conclusion: TGFαL3-SEB fusion protein was successfully designed, expressed and purified. This chimeric fusion protein exhibits potent in vitro antitumor activity. So, these results indicate that TGFαL3-SEB might be a promising anticancer candidate for cancer immunotherapy and further efforts are needed to explore this potential therapeutic strategy.

Keywords: Breast Cancer, Staphylococal Enterotoxin Type B (SEB)

OPTIMIZATION OF BENZENE BIODEGRADATION USING STREPTOMYCES ISOLATED FROM TABRIZ REFINERY

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Background: *Streptomyces* bacteria isolated from soil of Tabriz Refinery was used for biodegradation of benzene. Then kinetic modeling of biodegradation and optimization of environmental conditions and pH were performed.
Methods: After isolation and screening, effective species for degradation of benzene were detected. Then bacteria were cultured in Muller Hinton Agar. Mineral medium was used for experiments. In addition to benzene, bacteria were added to one of the samples and by comparison of mineral medium containing only benzene (control environment); the changes resulting from the influence of bacteria were investigated. The ability of bacteria to decompose benzene was determined by maximum wavelength using spectrophotometer. Further experiments were carried out using gas chromatography. After extracting experimental data, the reaction kinetic model for biodegradation was proposed and optimization of the concentration, temperature and pH was performed.
Results: Bacteria can play an important role in bioremediation and cleaning hydrocarbon contaminated soils due to their ability for biodegradation of benzene. This bacterium is capable of decomposing benzene up to 70% while this method does not produce harmful excipients. According to the experimental data and proposed kinetic model, reaction obeys a first-order-reaction kinetic. Optimal conditions include: PH 5; temperature 25 C.
Conclusion: The identified bacterium has an acceptable performance in biodegradation of benzene and can degrade it up to 70%. On the other hand, *Streptomyces* is indigenous and resistant to environmental and can be used in these fields with variable climate changes. The effect of *Streptomyces* on biodegradation of other aromatic hydrocarbons can be studied for further researches.

Keywords: Benzene, *Streptomyces*, Biodegradation, Reaction Kinetic Model, Optimization

PURIFICATION AND DETERMINATION OF OPTIMUM PH AND TEMPERATURE OF INTRACELLULAR L-ASPARAGINASE FROM BACILLUS SP.PG-02

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Background: Bacterial L-asparaginases are amidohydrolases that catalyze the conversion of L-asparagine to L-aspartate and ammonia. The enzymes isolated from *E. coli* and *Erwinia carotovora* are now being used in the treatment of acute lymphoblastic leukemia. However, prolonged administration of L-asparaginase causes an anaphylactic shock or neutralization of the drug effect. Therefore there is a continuing need to screen newer organisms in order to obtain strains capable of producing new and high yield of L-asparaginase. In the present study a novel strain, *Bacillus* sp.PG-02 was explored for the production of intra-cellular L-asparaginase.
Methods: *Bacillus* sp.PG-02 was grown in a modified M9 medium and incubated in a rotary shaking incubator. After 24 h of inoculation, the cells were removed by centrifugation. Sonication of the cells was carried out to release the intracellular L-asparaginase and DEAE-Cellulose Ion exchange chromatography with discontinuous gradient of NaCl was used for purification of enzyme. The optimum pH and temperature for L-asparaginase activity were determined. The molecular weight of the purified enzyme was estimated by SDS-PAGE.
Results: The bacterial strain, *Bacillus* PG-02 was isolated from the Persian Gulf sediments and screened for the ability of L- asparaginase production. The intracellular enzyme was then purified through DEAE column. The enzyme was eluted in 0.2 M NaCl. SDS-PAGE analysis showed that the enzyme was purified and its molecular weight was approximately 35 kDa. Enzyme demonstrated the maximum activity at pH 7.5. The optimum temperature of the activity of the enzyme was found to be 40 °C.
Conclusion: The purified L-asparaginase produced by *Bacillus* sp.PG-02 showed a good activity near the physiological condition. Thus, it is a potential candidate for medical applications.

Keywords: L-Asparaginase, *Bacillus*, Purification, Activity

SURFACE PROTEOM EXTRACTION OF BORDETELLA PERYUSIS AND IDENTIFICATION OF IMMUNOGENIC PROTEINS WITH DOT AND WESTERN BLOTTING

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Background: In this study, the analysis of well-known surface and secret immunogenic proteins of *Bordetella pertussis* in a standard reference and a vaccinal strain was performed. Surface and secret proteins of two strains were identified using different methods of sample preparations and extractions along with analysing immunogenicity by Dot and Western blotting.
Methods: Bacterial pellet was treated with 3M urea and centrifuged at 26,000g for 15 min. The supernatant was recentrifuged (26,000 g,r 1 h) and used as an outer membrane protein. Harvested bacterial cells were resuspended in 10 mM sodium phosphate (pH 7.2) and disrupted on ice using a cell sonicator. The supernatant after spinning was removed and centrifuged (100,000 g, 4°C, 1 h). The resulting pellet was resuspended in phosphate buffer containing 0.5% sarkosyl. The mixture was shaken and centrifuged to pellet the outer-membrane proteins. The outer membrane-enriched fractions were suspended in phosphate buffer and used as outer membrane protein. SDS-PAGE electrophoresis was applied based on Laemmli method in a 10% separating gel. Dot blot and western blot analysis were carried out with outer membrane enriched fraction using antibodies against the main components of bacteria and membranes were probed with a second conjugated-HRP antibody using DAB as substrate.
Results: Well-known surface proteins of *B. pertussis* Tohama I and vaccine strains were determined to obtain an insight into the protein distribution of the organism during growth phases. Different approaches and modifications were employed to isolate OMPs. The Sarkosyl extraction method was more appropriate than the urea method for this organism in terms of the total number of outer membrane proteins obtained on the gel. Dot blot analysis followed by 1D-SDS-PAGE then visualized by staining and lastly Western blotting was performed. Most of sodium phosphate well-known virulence factors of *B. pertussis* strains such as pertussis toxin (PT), filamentous hemagglutinin (FHA), fimbrial subunits (Fim1, Fim2, Fim3) and pertactin (PRN) were identified during growth phases in bioreactor.
Conclusion: Our findings are expected to facilitate surfacetome and secretome analyses of *B. pertussis* including physiological proteome and aid the manufacturer in terms of producing more potent vaccines based on changes in process of development and in progress of new vaccine.

Keywords: *Bordetella pertussis* - Outer Membrane Protein - Immunogen - Western Blot

DESIGN AND CLONING OF RECOMBINANT PEPTIDE DRUG GENE, TERIPARATIDE IN E.COLI BL21(DH5A)

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Background: The aim of this research is to design and to clone the rhPTH gene in *E. coli* BL21(DE3).
Methods: This research discloses a novel frame designed for efficient preparation of N-terminal 1-34 amino acids of hPTH as functional parts of this hormone. The frame constructed as an NcoI-BamH1 fragment encoding a His-tag and a chimeric fusion protein consisting of a fusion partner comprising of 52 amino acids belonging to *Escherichia coli* β-galactosidase (LacZ) gene, a cleavage site identified by Entropeptidase and rhPTH (1-34) gene fragment. Optimized frame was synthesized and ligated with pET28a vector under the control of T7 promoter, and then transformed in *E. coli* BL21(DH5α) cells.
Results: Positive clones that released the mentioned frame by double digestion with NcoI-BamH1 enzymes were approved by sequencing.
Conclusion: We cloned designed gene in *E. coli* Bl21(DH5α). We expect to express our designed gene to be very elevated after itsinduction by IPTG in *Escherichia coli* BL21 (DE3).

Keywords: *E. coli*, Teriparatide, Osteoporesis, Rhpth- Cloning

MOLECULAR DETECTION OF MICROCYSTINE PRODUCING IN WATER OF PERSIAN GULF, HORMOZGAN PROVINCE WITH PCR METHOD

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Background: This study aimed to diagnose the molecule which produces *Microcystine* gene in Cyanobacterias of Persian Gulf in Hormozgan using PCR method.
Methods: A paire of primers, PC-R and PC-F, were used to diagnose Cyanobacterias and other paire of primers, Mcya-Cd1R and Mcya-Cd1F were used for diagnosing the gene which produced Toxin Microcystine A mcyA. Then, PCR test was gotten the optimum and allergy and specialty test were also done. The product of increased gene was transformed by plasmid Ptz257 in *E. coli* bacteria (JM1.7). 20 samples of water were prepared from various stations in Persian Gulf and consequently, by using transformed DNA methodology, DNG was extracted and the presence of Cyanobacteria and Cyanobacterias generating Toxin was investigated.
Results: Optimums PCR for primers with the length of 650bp and Toxin Microcystine with the length of 297bp were increased and then they were observed by Electrophorese gell. Using specialty test by various primers with DNA, related to eight Microorganisms, proved 100% of their specialty. The presence of Cyanobacteria in all 20 stations was admitted but the presence of Cyanobacterias generating Toxin was admitted in three stations.
Conclusion: In diagnosing Cyanobacteria and the strains of Toxin generator, PCR methodology was faster and more accurate than traditional methods. This method can also be used to find the certain presence of the Cyanobacterias generating Toxin.

Keywords: Microcystine, PCR, Cyanobacteria, Persian Gulf

PRODUCTION OF PECTINASE BY ASPERGILLUS NIGER USING SOLID-STATE SUBSTRATES

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Background: The purpose of this research was to optimize the production of pectinase by solid substrate fermentation using lignocellulosic wastes (wheat straw, rice straw) and pectic substance such as citrus pulp (lemon, orange).
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 affecting the pectinase production by strain under study. The effect of addition of various solid-state substrates such as wheat straw, rice straw, orange pulp and lemon pulp, pulp ratio, carbon / nitrogen (C/N) ratio, and pH was studied for optimal pectinase production. Pectinase assay was done spectrophotometrically using galacturonic acid (GaiA) as the substrate.
Results: In present study mixed of wheat and rice straws was utilized in combination with orange and lemon pulp with various percentages to product the enzyme with Aspergillus niger. The results showed that the highest pectinase activity was obtained using lemon pulp and orange pulp with maximum activity of 7819.15 U/mg. In addition the maximum pectinase production in the pulp of oranges and lemons was obtained at C/N ratio of 2 and 1%, respectively. Also the highest enzyme activity in orange pulp and lemon pulp was found at PH=6.
Conclusion: The great amount of pulps daily produced during citrus juice processing makes their elimination difficult through a single system. Unprocessed pulps could be partially utilized as components of animal feed mixtures or source of human dietary fibers, whose world demand is increasing. Pulps could be alternatively utilized as carbon sources to grow microorganisms. Result indicated that increase for pulp as a solid substrate for the enzyme production was increased. This study showed that citrus pulp due to its high pectin is a potential source for increasing the production of pectinase.

Keywords: Pectinase; *Aspergillus niger*; Solid-State Fermentation; Citrus Pulp

SCREENING OF ACTINOMYCETES FROM LIPAR AREA OF GULF OF OMAN TO RESEARCH THE ANTIMICROBIAL COMPOUNDS

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Background: Actinomycetes are one of the most important sources for producing antimicrobial compounds and the most important prokaryotic microorganisms in this regard. Diversity of antimicrobial compounds isolated from terrestrial Actinomycetes has been declining and considering the increasing resistance of bacteria to antibiotics compounds, searching for new sources is necessary. Marine environment due to its unique characteristics is considered as a good option to search for bacteria with the capability of producing antimicrobial compounds.
Methods: To evaluate the antimicrobial compounds from Actinomycetes, water samples were obtained from the Gulf of Oman (Particularly, from Lipar area). To maximize the isolation of Actinomycetes, the process was done using starch-casein agar, Starch Nitrate Agar and Glycerol Glycine Agar with two different salt concentrations. To investigate the Antimicrobial production activity, the isolated Actinomycetes were assessed against reference and pathogenic gram positive strains like *Staphylococcus epidermidis, S.intermedius* and Methicillin resistant *S.aureus* and gram negative strains like Pseudomonas, Listeria, Klebsiella, Salmonella, *Acinetobacter* and *E. coli* O157 by cross streak method.
Results: 35 isolated cases belong to the *Actinobacteria*, based on morphological characteristics, pigment production and aerial mycelium and that %94 of these bacteria have the ability to produce antimicrobial compounds. Most of the isolated bacteria have antimicrobial compounds against reference *S.aureus* among gram positive bacteria and *Listeria* among gram negative bacteria. Inhibition zone is measured between 2 to 25 mm in diameters for garm positive bacteria and 1-20 mm in diameters for gram-negative bacteria.
Conclusion: Native Iranian *Actinobacteria* could be considered as suitable options to screen the new antimicrobial compounds. Molecular research and antimicrobial compounds extraction against the aforementioned pathogenic strains, are also being conducted.

Keywords: *Actinomycetes*, Antimicrobial Compounds, Lipar Area

THE SCREENING OF TELAR FOREST ACTINOMYCETES TO SEARCH THEIR ANTIMICROBIAL COMPOUNDS

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Background: Due to the emergence of bacterial resistance to antibiotics used, searching for new sources in order to make an important discovery of effective drugs is of great impportance. According to numerous studies, Actinobacterials are one of the largest groups of bacteria to produce the antimicrobial compounds. *Actinomycetes* are a unique group of bacteria that are widely distributed in natural ecosystems, particularly soil and have a special role in the production of secondary metabolites.
Methods: Sampling was performed from Telar forests of northern Iran. Isolation of *Actinobacteria* on casein-starch agar and glycerol-casein agar medium at 25 °C was performed. A total of 172 isolates were obtained, based on morphological characteristics the 55 isolates are belonging to Actinobacteria. To examine the ability to produce antimicrobial compounds, cross-culture method was used against reference and pathogen strains of Gram-positive bacteria such as *Staphylococcus epidermidis*, *S. intermedius* and methicillin-resistant *S.aureus* and gram-negative bacteria such as *Pseudomonas, Listeria, Klebsiella, Salmonella*, *Acinetobacter*, and *E. coli* O157 on Mueller Hinton agar. Antimicrobial effectiveness was evaluated by inhibition zone.
Results: 32 isolates of *Actinobacteria* have antimicrobial compounds. The predominant isolates have antimicrobial compounds against *S. saprophyticus* among Gram-positive bacteria and *Listeria* amonog reference gram-negative bacteria. According to the inhibition zone, 11 isolates produced the most antimicrobial compounds. 3 of them had activity against both positive and negative reference bacteria.
Conclusion: Primary results show the existence of *Actinobacteria* producing antimicrobial compounds in natural ecosystems. Evaluation of molecular characteristics of the isolates and extraction of antimicrobial compounds against the mentioned pathogenic bacteria are in progress.

Keywords: *Actinobacteria*, Antimicrobial Compounds, Telar Forests

EVALUATION OF PYRITE REMOVAL FROM MOUTEH REFRACTORY GOLD ORE USING ACIDITHIO BACILLUS FERROOXIDANS PTCC1647 IN SEMI INDUSTRIAL SCALE

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Background: The aim of this work is to evaluate the pyrite remove from mouteh gold ore by *A. ferrooxidans* PTCC1647 in column.
Methods: In the first step, Acidithio *Bacillus* Ferrooxidans PTCC1647 purchasad from (Iranian Research Organization for Science and Technology(IROST) was cultivated in the routinely 9k medium. In the second step, the cells suspensions (2x108 cells/ml) were used as inoculum at the 10 %v/v for column bioleaching. then column(75cm/7.5cm) was charged with 4Kg of sulfide gold ore from Mouteh gold mine isfahan province (Iran) with particle size 45 mesh(3.36 mm) containing FeS2(pyrite)78 %. 9K medium with initial pH 1.80 Containing pure cultures of A. Ferrooxidans PTCC1647 was pumped from the feed container to the top of the column. Air was pumped from Top of the column into holes embedded within the column. Experiments were carried out at 30 ºC for 60 days with pumping speed 1.8 l/h. Moreover, sterile column was prepared as the uninoculated control system. Redox potential, pH, ferrous iron concentaration(by spectrophotometer using 1, 10 orthophenanthroline ferrous complex as an indicator) and Sulfate concentration(indirectly determined by atomic absorption spectroscopy analysis of Ba after precipitation of BaSO4) were measured daily. The chemical composition and particle size distribution of ore was determined after bioleaching experiments.
Results: *A. Ferrooxidans* reduced the amount of ferrous ion form from 18.9 to 0.45 g/L. A decrease in pH (due to the oxidation of sulfide and sulfuric acid) and the concentration of Fe2 + (ferric iron due to oxidation) was initiated on the 14th day, and then continued until day 60. Ferrous iron to ferric iron completely was oxidized in 60 days. In chemical control flasks, only a negligible amount of ferrous iron was oxidized due to air-oxidation under the same experimental condition. XRD analysis showed that mineral pyrite was removed from ore after 60 days.
Conclusion: As result of long-term processes,a simultaneous use of these bacteria will give a better result. Since biological leaching processes in a semi-industrial experiment is considered as an introduction for industrial processes, the results of this research can be used in bio-oxidation of Mouteh refractory gold ore by industrial methods.

Keywords: Biolaching, Refractory Gold, Column

ISOLATION AND CHARACTERIZATION OF PHENOL- DEGRADING BACTERIA FROM THE ARAK PETROCHEMICAL COMPLEX

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Background: Phenol is an environmental pollutant present in industrial wastewaters such as refineries, coal processing and petrochemicals industries. Bioremediation has become one of the most rapidly developing fields of environmental restoration, utilizing microorganisms to reduce the concentration and toxicity of various chemical pollutants. This approach represents a good alternative compared with physicochemical strategies, which have high costs and produce other toxic products.
Methods: Phenol degrading bacteria were isolated from activated sludge of Arak Petrochemical Complex by serial dilutions of enriched consortium. In order to assess the utilization rates of phenol using the isolated strains, microbial inoculums were passed to the mineral salt medium with phenol (1000mg/l) as the sole carbon and energy source. The efficiency of isolated strains was assessed by monitoring the bacterial growth (OD600nm) and phenol biodegradation (spectrometrically using 4-aminoantipyrene). The experimental cultures were performed at 30°C at 140 rpm for 7 days.
Results: Among the isolated strains, NS1 had the highest rate of growth. After 7 days its degradation was approximately 70%.This strain was round-shaped, forming small flat orange colonies (on nutrient agar medium), gram positive, oxidase negative and catalase positive.
Conclusion: Isolated bacteria from Arak Petrochemical Complex have potential to degrade phenol. Also, successful biodegradation efficiency of the isolated strain may be accomplished during the process of optimization.

Keywords: Bacteria, Bioremediation, Phenol

EVALUATION OF CRUDE OIL BIODEGRADATION AND LACCASE ACTIVITY IN NEWLY ISOLATED PHAEOSPHAERIA SP UTMC 5003.

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Background: The present study was carried out to investigate Iranian indigenous fungal strains to remove crude oil pollutants.
Methods: First, for the fungal strain isolation, the contaminated soil samples were collected from different habitats of Iran. The soil samples were enriched in Minimal Salt Medium (MSM) supplemented with 1% crude oil, and chloramphenicol. Purification of isolates was carried out on PDA media. Afterward, each of pure fungal isolates were evaluated for growth rate and degradation ability after 15 days under 1% crude oil in MSM, by measuring the dry weight, total petroleum hydrocarbons (TPH) assay at 420 nm and the residual hydrocarbon content by FTIR. In the following, laccase activity of this isolate in the presence of 1% crude oil was measured. Finally, the best isolate was identified based on 18s rRNA gene sequence analysis and its morphology of colony and microscopic examination with the reference to identification keys.
Results: 30 different fungal strains were isolated. TPH test and gravimetric growth of each isolate in MSM supplemented with 1% crude oil as the sole carbon source showed that the isolate G-05 with 65% degradation over 15 days, was the best isolate in removing the petroleum hydrocarbons. Residual crude oil analysis with FTIR spectrophotometry in PDA+1% crude oil medium also indicated that G-05 is able to degrade 90% of aliphatic compounds. Tensiometric assay showed that G-20 is a potent strain for bio-surfactant production. Evaluation of laccase activity showed that this isolate can produced 1440 U/l of enzyme at the end of 15 days. Molecular and morphological identification indicated that G-05 is *Phaeosphaeria* spp.
Conclusion: In spite of high potential of fungi in bioremediation of contaminants such as crude oil pollutants, few studies have been carried out in this field. The results of this study showed that this isolate that is being reported for the first time, has a high potential in bioremediation of the soils contaminated oil.

Keywords: Bioremediation, *Phaeosphaeria* Spp, Crude Oil, Environmental Biotechnology

AMYLASE PRODUCTION WITH BLACK SUGAR DURING SUBMERGED FERMENTATION AND THE PROCESS OF OPTIMIZATION BY BACILLUS SPP.

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Background: The purpose of the present study was investigation of amylase production using black sugar by submerged fermentation method and process optimization by members of the genus *Bacillus*.
Methods: Optimization of different factors such as time, pH, shaking, and inoculation of amylase enzyme on production in submerged culture using RSM method were conducted.
Results: The maximum amount of production of experimental enzyme obtained in submerged culture at 24 hours, level of rpm 250, and pH=6, and inoculation rate of 20% was achieved.
Conclusion: Since black sugar is native for guilan province, and also in terms of price is affordable, it has been used in this study.

Keywords: Sugar Black, *Bacillus*, Alpha-Amylase, RSM

ISOLATION OF PHYTASE-PRODUCING BACTERIA FROM RHIZOSPHERE OF IranIAN WHEAT

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Background: In this study, phytase-producing bacteria from7 cultivar of rhizosphere wheat (Chamran, Tajan, Morvarid, Pishtaz, Oroum, Sivand, Zarea) were solated and identified based on morphological and biochemical characteristics.
Methods: Enzyme-producing bacteria were determined by production of clear zones around the colonies on the medium containing sodium phytate, after 24-48 h of incubation at 30°C. Because some bacteria in phytase specific medium are able to create false positive results,we eliminated them by phytase plate assay (cobalt chloride, ammonium molybdate / ammonium vanadate). The best phytase producing isolates were recognized by its surrounding clear halo.
Results: Out of 47 colonies on media plates, 19 colonies showed positive for phytase production and created zone of clearing around microbial colonies, but 11 false positive colonies were detected. Finally, 8 isolates were detected as real phytase-producing bacteria. Most isolated bacteria belong to two cultivars (Tajan, Chamran). One strain creating 23 mm zone was selected as the best enzyme producing isolate.
Conclusion: This research provided important data about phytase producing bacteria isolated from rhizosphere wheat, which will facilitate future research on the optimization of fermentatoin processes for production of high phytase activity.

Keywords: Phytine, Phytase, Phosphatase, Rhizosphere Wheat, Cultivar.

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DESIGN AND SYNTHESIS OF NOVEL DIPHENYLUREA DERIVATIVES WITH ANTIMICROBIAL AND ANTIFUNGAL POTENTIAL

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Background: As regards the halogen, atoms in the pharmacophore structure can be beneficial for antimicrobial activity; therefore, chloro derivatives were chosen to synthesize our target compounds. In view of these observations, we have designed new compounds incorporating with the above pharmacophores together in order to prepare molecules having enhanced antimicrobial and antifungal activity. Therefore, we synthesized new compounds containing urea groups in order to investigate their antibacterial and antifungal activities.
Methods: 4-amino phenyl ethyl ester 1 as a base compound was available. 4-amino phenyl hydrazide 2 was prepared by reaction of hydrazine hydrate with compound 1. 4-amino phenyl hydrazone 6, 7 was prepared by reaction of compound 2 with 3-chlorobenzaldehyde and 4-chlorobenzaldehyde. Target compounds were synthesized by the reaction of compound 6, 7 with corresponding isocyanat.
Results: Thin layer chromatography was used to determine the purity of the compounds synthesized. Definition of the melting points was determined in open capillary tubes for the presented Impurities. Then target compounds were reviewed and approved by the FT-IR and H-NMR spectroscopy based on the number of protons. The activity of target compounds about the protein binding sites was evaluated by using the outodock configuration software.
Conclusion: Because of the existence of both hydrazone and urea pharmacophores in the target compounds their antimicrobial and antifungal activity can be considerable. According to the results of spectroscopic and analytical data and a potent active part of urea; it is anticipated that the final composition for the purpose intended, has been accepted in the invitro tests for antibacterial and antifungal effect.

Keywords: Diphenylurea, Hydrazone, Antimicrobial, Antifungal

LIQUID–LIQUID EXTRACTION OF ALKALINE PROTEASES FROM FERMENTED BROTH BY PEG/SODIUM NITRATE AQUEOUS TWO-PHASE SYSTEM

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Background: The objective of the study was to determine extraction conditions include polyethylene glycol (PEG) (%35, 40, 45),salt concentration (%25, 30, 35), phase volume ratio (%0.5, 0.75, 1) and system pH (8, 9, 10).
Methods: In this study, *Bacillus* sp. was isolated and identified by Kamali Yazdi and et al. The initial culture was grown in 250 ml Erlenmayer flask containing glucose (10 g/l), pepton (5g/l), yeast extracct (5 g/l), Mgso4.7H2o (0.5% w/v) and the pH was adjusted (50mM carbonate-bicarbonate buffer)to 9.The flasks were incubated at 37 C on rotation shaker at a speed of 250 rpm for 24 hours. PEG 6000/Sodium nitrate ATPS of different pH values (8, 9, 10) were prepared at room temperature.In a sterile test tube, 5.0 ml of PEG 6000, 5.0 ml of Sodium nitrate and 2.0 ml of enzyme from the fermented bacterial were taken and undisturbed for 10 minutes at room temperature. This mixture was centrifuged at 5,000 rpm for 15 minutes. Top phase and bottom phase were collected and absorbance of sampels was measured at 280 nm.
Results: The best performance of the system was obtained applying 40 (% w/w) PEG 6000 concentration, 30 (%w/w) Sodiumnitrate concentration and 1(% w/w) phase volume ratio at pH 10 (119.88 U/ml).
Conclusion: Phase composition of the aqueos two-phase systems had a significant effect on enzyme partitioniong.The PEG/Sodium nitrate system was proved suitable for alkaline protease removal from fermented broth of *Bacillus* sp. Four successive experimental designs were used in the present study to select the best conditions of protease extraction by PEG/Sodium nitrate ATPS.

Keywords: Alkaline Protease, Aqueous Two-Phase System, Liquid–Liquid Extraction, *Bacillus* Sp

MALATHION BIODEGRADATION BY ISOLATED BACTERIA FROM ARVANDKENAR CONTAMINATED SOIL

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Background: Malathion is one of the organophosphorus insecticides which has a wide variety of applications in the agriculture industry. Its harmful toxic effects on animals range from invertebrates to vertebrates including human. Malathion can be degraded using physical, chemical or biological methods. Bioremediation is effective when compared to the other methods and applicable for insitu bioremediation. Present study reports the isolation, morphological and biochemical analysis of malathion degrading bacteria isolated from Arvandkenar contaminated soil.
Methods: Malathion degrading bacteria were isolated from Arvandkenar contaminated soil by serial dilutions of enriched consortium. In order to assess the utilization rates of Malathion using the isolated strains, microbial inoculums were passed to the mineral salt medium with malathion (2ml/l) as the sole carbon and energy source. The efficiency of isolated strains was assessed by monitoring the malathion biodegradation (using GC- FID). The experimental cultures were performed at 35˚C at 130 rpm for 7 days.
Results: Results showed that, among the isolated strains, BNA1 had the highest malathion biodegradation abilities (22.00%) in comparison to other isolated strains. This strain was short rod-shaped, forming cream colonies (on nutrient agar medium), gram negative, oxidase negative and catalase positive.
Conclusion: Isolated bacteria from Arvandkenar contaminated soil have the potential to degrade malathion. Also successful biodegradation efficiency of the isolated strain may be accomplished during the process of optimization.

Keywords: Organophosphorus, Biodegradation, Bacteria

RAPID IDENTIFICATION OF ALTERNARIA ALTERNATA FUNGI,THE CAUSE OF BROWN SPOT DISEASE BY LOOP-MEDIATED ISOTHERMAL AMPLIFICATION

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Background: Therefore, in this study rapid identification of *A. alternata* using Loop-Mediated Isothermal Amplification (LAMP), which is a precise and cost effective method, was developed.
Methods: Phytopathgenic *A. alternata* strains were isolated from infected citrus trees in Ramsar, Iran. Eight different strains were isolated in PDA and PCA media and were identified as *A. alternata* using PCR technique and ITS primer. A specific pair of LAMP primers was designed using ACT-toxin gene using PRIMER EXPLORER V4 software. Subsequently, LAMP reaction was performed with Bst DNA polymerase at constant temperature 60, 65 and 70 °C for 60 min. LAMP reaction was done on all isolated *A. alternata* in addition of 15 DNA extracted from standard fungi and bacteria, with an exception of *A. alternata* as a negative control and 5 DNA extracts samples from soil. Fluorescent dye SYBR Green and turbidimetry were employed to detect positive samples.
Results: According to the carried out reactions, all pathogenic isolated *A. alternata* and standard control showed green dye and turbidity caused by amplification of ACT-toxin gene in 60, 65 and 70 ºC. No amplification was detected in 75 ºC. In contrast, none of standard fungi DNA samples showed positive result in this reaction. Moreover, positive and negative results were observed for 2 and 3 soil samples, respectively. The obtained results showed that the LAMP designed primers for ACT-toxin gene as well as the introduced approach can be applied to detect the Phytopathgenic *A. alternata* with high precision in the short time.
Conclusion: Results presented in this study showed that ACT-toxin gene is highly specific and designed LAMP primers can be used in molecular diagnosis of *A. alternata* in DNA sample, which extracted from fungi and even from soil.

Keywords: Molecular Identification, Brown Spot Disease, *Alternaria alternata*

RHAMNOLIPID PRODUCTON USING SOYBEEN OIL REFINERY WASTES

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Background: Rhamnolipid type biosurfactant production by *Pseudomonas aeruginosa* MR01 was investigated using soapstok waste of soybeen oil refinery as a Low Cost carbon source. Physiochemical properties of rhamnoipid MR01 were studied to figure out the possibility of using this product in idustrial aplications.
Methods: In this research, the production yield of P. aeruginosa MR01 was investigated in different broths, each containing 2%,4%,6%,8%,10% and 12% (V/V) of soapstock. The production was purified using column chromatography.Biosurfactant emulsification was determined through the emulsification index (E24). And critical micelle concentration was measured by ring method.
Results: The best yield was obtained using 8% V/V of soapstock. The purity of the production was 89.28%. The MR01 rhamnolipids from soapstock showed good tensioactive properties with a minimal surface tension of 26.34 mN/m and a critical micelle concentration (CMC) of 59.25 mg/l. The MR01 biosurfactants formed stable emulsions with Kerosene and showed excellent emulsification of this substance (66.66%).
Conclusion: These results demonstrate the possibility of using soapstock as an excellent carbon source and the production can be useful for industrial applications, such as the bioremediation of oil spills

Keywords: Rhamnolipid, Biosurfactant, *Pseudomonas aeruginosa*

NUTRIENT AND PHYSICAL VARIABLES OPTIMIZATION OF PHB PRODUCTION BY ALCALIGENES EUTROPHUS

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Background: Polyhydroxyalkanoates (PHAs) are completely biodegradable thermoplastics, which are accumulated as an energy reserve material by a large number of bacteria in the presence of excess carbon source and limitation of nitrogen or phosphorous sources. In the present study, efforts have been made to optimize the effect of various nutritional and physical parameters for enhanced PHB production by *Alcaligenes eutrophus* PTCC 1615 using Plackett-Burman and Response Surface Methodology statistical methods.
Methods: In the present study, efforts have been made to optimize the effect of various nutritional and physical parameters for enhanced PHB production by *Alcaligenes eutrophus* PTCC 1615 using Plackett-Burman and Response Surface Methodology statistical methods. At first, based on one-factor-at-a-time experiments, fructose and ammonium chloride were found to be the most suitable sources of carbon and nitrogen for PHB production.
Results: Then through the Plackett-Burman and central composite design fructose, agitation speed, KH2PO4, and initial pH were recognized as the most significant factors affecting PHB accumulation. ANOVA analysis of the model showed a significant interaction between fructose and agitation speed.
Conclusion: After optimization of the medium, compositions for PHB production were determined as: fructose 45 g/L, KH2PO4 2.12 g/L, MgSO4.7H2O 1.2 g/L, citric acid 1.7 g/L, trace element 10 mL/L, initial pH =7.78 and agitation speed 198 rpm. Under this optimal culture conditions, the maximum yield of PHB was 9.41 g/L. These results are the highest values of PHB ever obtained from *A. eutrophus* reported so far.

Keywords: Biopolymers, PHA, Biodegradable, *Alcaligenes eutrophus*

STUDY OF ORGANOPHOSPHORUS INSECTICIDE CHLORPYRIFOS BIODEGRADATION BY HALO-TOLERANT BACTERIA ISOLATED FROM CONTAMINATED AGRICULTURAL LANDS

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Background: Because many agricultural lands in Iran are saline, aim of this project is to investigate the biodegradation of cloropyrifos as an Organophosphorus (OP) compound model by halo-tolerant bacteria isolated from agricultural land.
Methods: Soil and water samples were collected from saline agricultural areas with history use of OP. For isolation of bacteria capable of utilizing CP as sole carbon source, enrichment technique with the modified-M9 medium supplemented with 50 ppm CP and 5% NaCl was used. The time of each round of enrichment was 14 days. After three rounds of enrichment, modified R2A agar medium (with 5 % NaCl) was used for isolation and purification of single colonies. To select the isolates with higher resistance to CP toxicity and CP-degraders that are more efficient the growth of selected strains in modified-M9 media containing 100 to 500 ppm CP as sole source of carbon was assayed after 7 days with UV-visible spectrophotometer at 600nm. Plate-count method was used to compare the growth rate of the isolates in presence of 500 ppm CP.
Results: After three rounds of enrichment, 28 morphologically different bacterial colonies were isolated and purified. Evaluation of the growth of the isolates in the presence of 100 ppm CP after 7 days, led to selection of 5 isolates for further studies. Growth measurement of the selected strains in presence of 100 to 500 ppm CP revealed that the rate and extent of the growth of 3 strains CDB1, CDB2 and CDB3 have increased along with pesticide concentration, the results of plate count method also confirmed the increased growth of isolates in 500 ppm CP. Microscopic examination of these isolates indicated that all of the strains are Gram-negative bacilli.
Conclusion: Bacteria isolated in this study which are able to use CP as the sole carbon source for their growth, can be promising candidates to remove these compounds from contaminated brackish water bodies and because of structural similarity of OP pesticides, they can be helpful for biodegradation of other members.

Keywords: Organophosphorus (OP) Pesticide, Chloropyrifos, Biodegradation, Halotolerant Bacteria

STUDY OF AMYLASE-PRODUCING BACTERIA BIODIVERSITY IN THE DRILLING FLUID

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Background: Biodegradation of starch used in drilling fluid by many microorganisms, results in reduction of starch concentration in the fluid, which leads to fluid efficiency downfall. The aim of present study was to identifythe major starch degrading bacteria in the drilling fluid and manner of controling the growth and activity of these organisms.
Methods: Bacteria present in back up drilling fluid, were isolated and their amylase activity was studied by starch hydrolysis test. The strain with the most ability of starch hydrolysis in all three temperatures (37, 45 and 55°C) was selected as the superior strain and was identified by 16Sr RNA. Also growth curve of superior strain was plotted.
Results: 54 bacterial strains include 26 Gram-positive Bacillus, 22 Gram-negative *Bacillus* and 6 Gram-positive Cocci were isolated. 18 strains from 26 Gram-positive *Bacillus* were spore forming bacteria. Three strains had growth in all three temperatures (37, 45 and 55°C) and showed significant amylase activity. Bacteria with the most mean of inhibition zone were selected as superior strains. The results of the 16Sr RNA analysis showed 99.8% similarity of superior strain to *Bacillus* licheniformis. The bacterium was in logarithmic phase at 8-48 hour after inoculation.
Conclusion: Spore forming Gram-positive *Bacillus* belonging to *Bacillus* Genus like *Bacillus* licheniformis isolated in this study was the most active bacteria of degrading in well special conditions. Identification of degradation factors with the aim of selecting a suitable biocide is important to prevent the biodegradation.

Keywords: Biodegradation, Drilling Fluid, Starch

QUANTITATIVE ASSAY OF ANTITUMOR ENZYMES FROM HALOPHILIC AND HALOTOLERANT BACTERIA

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Background: Halophilic bacteria may contain antitumor enzymes with novel immunological properties that can be used in hypersensitive patients.
Methods: In former investigation, qualitative screening for L-asparaginase, L-Glutaminase and L-Arginase had been done. In the next step, enzymes activity was measured during growth curve plotting of positive strains. For this purpose, 1.5 ml of each positive strain inoculum (0.5 McFarland standards) was added to 50ml broth medium (supplemented with 2.5% total salts and substrate) and incubated in shaker incubator (150 rpm, 34 ° C). During their growth curve plotting, quantitave assay of the enzymes was measured by Nessler's reagent (For L-glutaminase and L-asparaginase) and ninhydrine (For L-arginase).
Results: Among 115 strains, 33, 7 and 5 strains were positive in L-asparaginase, L-glutaminase and L-arginase production, respectively. Activity of all the enzymes was increased during logarithm phase and maximum activity (IU/ml) was observed in stationary phase, especially in the end of stationary phase. *Bacillus* sp. strain R2S25 (7.5 IU/ml), *Bacillus* sp. Strain R2S12 (0.27 IU/ml) and *Planococcus* sp. Strain GAAy3 (5.5 IU/ml) respectively demonstrated maximum activity in L-asparaginase, L-gltaminase and L-arginase, respectively. 1 I.U (International Unit) of the enzyme is equal to amount (µl,pmol) of product produced from enzyme per minute.
Conclusion: This investigation showed that the halophilic and halotolerant bacteria from different hypersaline environments in Iran are a potential source of antitumor enzymes and may have possess commercial value.

Keywords: Antitumor Enzyme, Halophilic Bacteria, L-Asparaginase, L-Glutaminase, L-Arginase

INVESTIGATION OF DIFFERENT MICROALGAE CELL DISRUPTION METHODS

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Background: Cell disruption processes are important pretreatment techniques for extraction of intracellular contents from microalgae. In this study we tried to compare the results obtained for different cell disruptions methods by calculating the total chlorophyll content
Methods: We have evaluated six different methods; ultrasonic, osmotic shock, microwave, bead mill, liquid nitrogen and centrifuges as the cell disruption methods. 0.2 grams of dry powders of *Chlorella vulgaris* are mixed with 30 ml 90% methanol and after cell disruption, samples were kept at -20°c for 24 hours.. Finally total chlorophyll content was calculated according to the data obtained
Results: By comparing the results obtained for the total chlorophyll contentrespectively ultrasonic methods(7.50mg/l), bead mill(6.38mg/l), osmotic shock(4.50mg/l), microwave (4.11mg/l), centrifuges (3.21mg/l) and liquid nitrogen (3.01mg/l) had the best performance in the destruction of the cell wall
Conclusion: Although ultrasound is the highest efficient in the disruption of cell wall, but this method and microwave due to the high cost of energy consumption can be used only in laboratory studies and osmotic shock due to excessive time required (48hr) is not appropriate. In industrial scale, bead mill due to high efficiency and less cost of energy consumption is suggested.

Keywords: Chlorophyll - Cell Disruption- Microalgae

UTILIZATION OF DATE PALM AS A SUBSTRATE FOR ETHANOL PRODUCTION BY YEASTS ISOLATED FROM DATE PALM

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Background: The aim of this study was ethanol production from date palm by yeasts isolated from date palm.
Methods: Isolation of yeasts was done according to KIran Sree et al. (2000). For juice preparation from date palm, 100 grams of Zahdi date were mixed with 1 liter distilled water and after mixing them, they were filtered through filter paper. The obtained filtrate was autoclaved in 121°C for 15 min. 1% of 24-hour culture of yeast was used as the inoculum. Determination of ethanol content was done based on dichromate potassium procedure by some modification.
Results: In the result 5 isolates including D1, D2, D3, D4 and D5 were isolated from date palm. Among these isolates, D4 was the best isolate and produced 4.9% and 3.2% ethanol in aerobic and anaerobic conditions, respectively. Results of assimilation and fermentation tests of carbohydrates showed that this isolate is *Pichia burtonii*.
Conclusion: In conclusion, this isolate can be used for ethanol production in large scales. Although the ethanol yield is low, maximum being, 4.9% produced by *Pichia burtonii* D4, the strain can produce more ethanol by optimization of culture conditions.

Keywords: Ethanol, Date Palm, Dichromate Potassium

CONTINUOUS SCREENING OF LIPID DEGRADATING YEASTS AND ITS APPLICATION IN TREATMENT OF OILY WASTEWATER

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Background: The aim of this study was to continue the screening of lipophilic yeasts with high specific growth rate for wastewater treatment.
Methods: Soil samples were collected from soil with oil pollution. These samples were used in a continuous screening system in which fresh medium was poured in the main container as feed and the waste was removed in periodically time scale. Samples were taken from the main container each day and cultured in PDA plus chloramphenicol (100 mg/L) and Rose Bengal. A synthetic wastewater containing 10 g of olive oil, 0.6 g of peptone, 0.4 g of beef extract, 0.1 g of urea, 0.1 g of Na2HPO4, 0.03 g of NaCl, 0.014 g of CaCl2, 0.014 g of KCl, and 0.01 g of MgSO4 per liter was used as the representative of lipid containing wastewater. This medium was incubated at 28 °C in rotary shakers and the lipid degradation rate was investigated with standard n-hexan oil extraction method for 3 days.
Results: In this study, continuous screening was used to isolate the most predominant yeast capable of degradating lipids with high efficiency and growth rate. The obtained isolate was identified by 18S rRNA gene sequence analysis as *Rhodotorula mucilaginosa* (UTMC 5004). The entire isolates were preserved in University of Tehran Microorganisms Collection (UTMC). The oil degradation of the *R. mucilaginosa* reached 67 % in 3 days with 6.8 % wet cell weight
Conclusion: Although lipid-degradading bacteria have been examined exceedingly, yeasts have been less studied in terms of their great potentials in degrading lipids. These results indicate that *R. mucilaginosa* UTMC 5004 can be used in biological treatment of lipid containing wastewater and can be further investigated for the optimization of the process.

Keywords: Lipid Degradation, *Rhodotorula mucilaginosa*, Continuous Screening, Wastewater Treatment.

CYTOCIDAL ACTIONS OF PARASPORIN, AN ANTI-TUMOR CRYSTAL TOXIN FROM BACILLUS THURINGIENSIS

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Background: Parasporin, a new crystal protein derived from non-insecticidal and non-hemolytic *Bacillus thuringiensis*, recognizes and kills human colon cancer cells as well as some classes of human cultured cells. Here we report that a novel cytotoxic protein was isolated from the crystal produced by *B. thuringiensis* subsp. thuringiensis S9 strain.
Methods: Fifty soil samples were collected from different Iranian provinces, and characterization was performed based on protein crystal morphology by phase-contrast microscope and variations of Cry protein toxin using SDS-PAGE. After parasporin process with proteinase K, the active form was produced and protein activity on the cell line was evaluated.
Results: Parasporal inclusion proteins showed different cytotoxicity against human colon cancer cell line (HCT-116), but not against normal lymphocyte. Isolated parasporin demonstrated no hemolytic activity against human erythrocyte. It appears that these proteins have the ability to differentiate between normal lymphocytes and leukemia cells and have specific receptors on specific cancer cell lines.
Conclusion: The 37-kDa protein from S9 exhibited various degrees of cytocidal activity toward human colon cancer cells and caused cell swelling or the formation of blebs in the surface of the cells. Thus, parasporin acts as a cytolysin, which targets cell specificity and subsequently induces cell decay.

Keywords: *Bacillus thurngiensis*, Cry Protein, Cytocidal Activity, Human Cancer Cell.

ENDOGLUCANASE PRODUCTION BY BACILLUS CIRCULANS STRAINS GU25 AND GU38

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Background: The aim of this study was to evaluate the ability of β-1,3-endoglucanase production by *B. circulans*.
Methods: *Bacillus circulans* was cultivated in YMB media. In the next step, *B. circulans* was added to CMC (carboxy methyl cellulose) culture. The protein concentration and endoglucanase activity were measured.
Results: The optimal pH and temperature for high endoglucanase activity in the CMC culture were pH 5.5 and 40°C respectively. Under these conditions, isolate Gu38 with 3 × 10 -13 μmol/min/ml showed the highest endoglucanase activity in this temperature.
Conclusion: This study led us to conclude that *Bacillus circulans* strain Gu38 may serve as good source of endoglucanase production.

Keywords: *Bacillus circulans*, Endoglucanase, Carboxy Methyl Cellulase, Enzyme Activity.

STUDY OF PRODUCTION RATE OF LACTIC ACID IN COCCI STRAINS ISOLATED FROM DAIRY PRODUCTS OF Iran AND MOLECULAR AND CHEMICAL IDENTIFICATION OF PREFERABLE STRAINS

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Background: This study has been conducted to screen local cocci strains isolated from traditional dairy in order to select the superior strains in terms of lactic acid L(+) production.
Methods: In the first step, screening of 63 local cocci strains in terms of lactic acid production rate was performed by titration method. To make sure of the results, the titration was performed in three repetitions from strains with higher lactic acid production rate. Cocci strains producing lactic acidat a high rate for which the production had been repeatable were evaluated in enzymatic method. Superior strains producing lactic acid L (+) isomer were biochemically identified by eighteen-hydrocarbon fermentation. Then the superior strains DNAs were extracted and their 16s rRNA gene was amplified and after determination of sequence, the strain identities were obtained in comparison with information available in genome bank.
Results: Variation in production rate of lactic acid among local cocci strains was studied and significant variations were observed. In the first stage the highest level of lactic acid production obtained 27.25 mg/gr and the lowest obtained 13.50 mg/gr. The amount of producing pure lactic acid L (+) in the selected superior strains by enzymatic method was reported to be 3.35, 3.05 and 2.97, respectively. The two superior strains *Enterococcus* faecium and pastori staphylococcus were detected. 16S rRNA gene of the superior strains was reported in NCBI (the US National Center for Biotechnology Information) with access No. KJ503199, KJ508200 and Kf735653.
Conclusion: Cocci strains are of the most remarkable industrial spices to produce lactic acid for their hemofermentative feature. In this study production of lactic acid L(+) by *Enterococcus* faecium and pastori staphylococcus strains was reported higher than that of the previous reports. By screening these strains in terms of lactic acid L(+) production, valid strains were obtained which can be used in production of pure lactic acid with lower total cost for applications in pharmaceutical and medical industries

Keywords: Screening, Cocci, L(+)Lactic Acid, Microbiology, PCR

THE ROLE OF BACTERIAL INFECTION IN DEVELOPMENT OF MENTAL DISORDERS.

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Background: So far, there is no report considering Meta analysis study on microorganism and mental disorders. The purpose of this study was to investigate the psychological effects caused by bacterial infections.
Methods: In the current Meta-analysis study, we used keywords such as mental disorders,mental diseases and microorganism/depression and search engines SID, Iranmedex, Web of Science, PubMed and Google scholar. We did comprehensive data collection. All articles with no row data were excluded. According to inclusion, criteria out of 24 articles18 studies data were analyzed using Meta analysis software.
Results: Considering 34 studies, overall 7041 patient with diverse mental disorders and control have been studied. In these populations, *Chlamydia* spp, *E. coli*, *Brucella* spp. *Streptococcus* group A, *Mycoplasma* spp, and Mycobacterium, Helicobacter pylori have been studied. *Mycobacterium* spp. (33% case vs. 9.8% control), *Streptococcus* group A (38.% case vs. 14.6% control), *E. coli* (17% case vs. 4.5% control), *Brucella* spp,(24% case vs 10.2 control) and *H. pylori* (16.5% case vs. 5.2 control) contributed to development of mental disorders while *Chlamydia* spp (13.8% case vs. 13.6% control) did not show any association with psychological disorders. The most mentioned diseases contributing to these microorganisms were schizophrenia, depression and autisms.
Conclusion: According to the psychological impact of the disease on people lives, the role of bacteria in causing these diseases is very important as they can transmit from person to another. Considering the role of *Mycobacterium* spp, *E.coli*, *Brucella* spp. *Streptococcus* group A, and *H. pylori* in development of mental diseases, we suggest that an early detection and a rapid elimination of the bacteria can be helpful in prevention of mental illness.

Keywords: Mental Disorders, Bacterial Infection, Schizophrenia, Depression And Autisms

EVALUATION OF MICROBIAL LIFE IN SALT LAKE RAZAZEH, KARBALA – IRAQ

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Background: The aim of this research was to evaluate halophilic bacterial diversity in Lake Razazeh as regards this is the first report on halophilic bacteria isolation in Iraq.
Methods: Samples of saline soils, mud and water were collected from 15 locations of Lake Razazeh with distance of 4 km from each other. Halophiles were isolated by saline nutrient broth enrichment. The growth was diluted 10 times and plated on complete medium agar with a salt gradient of 2.5 percent. Cultures were incubated at pH 7-7.5 and 37°C during 7 days given that all medium were controlled every day. Gram staining was carried out as a routine initial procedure in the identification of unknown bacterial species.
Results: Two hundred and eighteen isolates were selected from isolation plates, which were named K1-K218. There were 161 gram-positive rod and cocci and 57 gram-negative rods. More than fifty strains were isolated using the morphological differences based on visible examination of the growth characteristics, and were used for further analysis. Also, 15 protozoa, 3 algae, 5 fungi and yeast were observed in the culture medium. It was found that the bacteria diversity in different media varied considerably so that only one type of bacteria grow in the 3 medium which likely showed these three bacteria are antibiotic-containing. About two third of the isolates were halotolerant and only eighteen isolates grew at NaCl concentrations greater than 17.5%.
Conclusion: The data presented here show that despite drought, dehydration, increased concentrations of salts and contaminants, Lake Razazeh near Karbala represents an untapped source of halophilic bacteria biodiversity.

Keywords: Biodiversity, Halophiles, Screening

THE STUDY OF ANTIBACTERIAL EFFECTS OF WATER AND ALCOHOLIC EXTRACT OF CLITOCYBE BRUMALIS MACROSCOPIC FUNGI

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Background: Fungi are groups of eukaryotic heterotrophy microorganisms. Today, the use of fungi extract as an antibacterial additional has an important role in protectingthe quality of food. In this experimental study antibacterial effects of water and alcoholic extract of Clitocybe brumalis species have been studied after extraction Invitro conditions on three soush Hos bacterias (*Salmonella* tiphymorium,, Proteus volgaris and *Staphylococcus epidermidis*).
Methods: In this study, effects of extract concentrations (0.1, 0.2, 0.3 mg/ml) were investigated using the disk diffusion method on bacteria *Salmonella* tiphymorium, Proteus volgaris and *Staphylococcus epidermidis*.
Results: The results presented that certain concentrations of fungus extract has an antimicrobial effect on microorganisms. Effect of alcoholic extracts decreased with their decreasing concentration, but the opposite was observed in the case of water extract.
Conclusion: The results of research show that the extract of macroscopic fungi *Clitocybe brumalis* has an antibacterialeffect. In this way we can hope that we will use this extract instead of chemical antibacterial drugs in order to treat bacteria infection. They will be better than chemical drugs which have many adverse effects.

Keywords: Macroscopic Fungi, *Clitocybe Brumalis*

THE STUDY OF RHIZOPOGON ROSEOLUS MACROSCOPIC FUNGUS ANTIMICROBIAL EFFECTS

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Background: Fungi are groups of heterotrophic microorganisms eukaryotic. Today, the use of fungi extract as an antibacterial additional has an important role in protecting the quality of food. In this experimental study antibacterial effects of water and alcoholic extract of *Rhizopogon roseolus* species have been studied after extraction Invitro conditions on three pathogenic bacteria (*Salmonella tiphymorium, Proteus volgaris* and *Staphylococcus epidermidis*).
Methods: In this study, effects of extract concentrations (0.1, 0.2, 0.3 mg/ml) were investigated using the disk diffusion method on bacteria *Salmonella* typhimorium, Proteus volgaris and *Staphylococcus epidermidis*.
Results: The alcoholic extract of *Rhizopogon roseolus* fungus has the greatest influence on bacterial species *Salmonella* typhimorium (14.8, 18.1, 19.6 mg/ml). However, the water extract has no effect on bacterial species.
Conclusion: The alcoholic extract of macroscopic fungi *Rhizopogon roseolus* has antibacterial effect. In this way we can hope that we will use this extract instead of chemical antibacterial drugs in order to treat bacteria infection. They will be better than chemical drugs which have many adverse effects.

Keywords: *Rhizopogon roseolus*, Macroscopic Fungi, Anti Microbial.

ANTIBACTERIAL ACTIVITY OF BASIL (OCIMUM BASILICUM) ESSENTIAL OIL AGAINST STAPHYLOCOCCUS AUREUS IN WATER BUFFALO MINCED MEAT

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Background: The present study aimed to evaluate the antimicrobial activity of basil essential oil against *Staphylococcus aureus*. The data show that basil oil was active against *S. aureus* with minimal inhibitory concentrations (MICs) ranging from 64-256 µg/gr.
Methods: In this research, the antimicrobial activity of Ocimum basilicum essential oil were investigated with different concentrations (0/001, 0/005 and 0.01%) on *Staphylococcus aureus* total viable count in minced meat at different time intervals: after treatment (day 0) and after storage for 15, 30 and 45 day at -12°C. All microbiological analyses performed for 3 times.
Results: The results showed that *Staphylococcus aureus* in all samples with different concentrations of basil essential oil, decreased during storage. This antimicrobial effect was significant on day 15 for *Staphylococcus aureus*. Our data also showed that the basil essential oil at 0/01% concentration caused reduction of *Staphylococcus aureus* in samples.
Conclusion: These data indicate that basil essential oil can exhibit antimicrobial activity against *Staphylococcus aureus*. So it can be considered as an alternative natural preservative in food products.

Keywords: Basil Essential Oil -*Staphylococcus aureus* - Water Buffalo Minced Meat

EFFECTS OF BUFFER ON THE KINETIC BEHAVIOUR OF BACTERIAL ENZYME: XANTHAN LYASE

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Background: The aim of this study isthe investigation of type of buffer (as an environmental factor) on xanthan lyase activity, a xanthan-degrading enzyme from Paeni *Bacillus* SP.SM0.
Methods: In the present study, bacterial xanthan lyase was cultured in fluid culture media. Supernatant was collected and activity of the enzyme was measured. Xanthan lyase activity was monitored by measuring the increase of A235 caused by conjugation of the formed C=C bond with the carboxylate group in the uronic acid residue.
Results: The enzyme saturation curve (Michaelis-Menten plot) was plotted in increasing concentrations of xanthan (substrate) in the presence of both phosphate and tris-HCl buffer. Vmax and Km of the enzyme were measured and comprised.
Conclusion: According to the parameters, it can be deduced that buffer has an effect on xanthan lyse activity and tris-HCl buffer is more suitable for a better activity of xanthan lyase.

Keywords: Polysaccharide, Xanthan Lyse Activity, Buffer

SCREENING FOR URICASE ENZYME FROM HALOTOLERANT BACTERIA.

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Background: Uricase (urate oxidase, EC 1.7.3.3) is the enzyme that catalyzes specifically the oxidation of uric acid to allantoin and plays an important role in nitrogen metabolism. Uric acid and its salts are relatively insoluble in water, easily precipitated, and the abnormal accumulation of uric acid is a causative factor of gout in humans. The aim of this study was screeinning for new resoureces for uricase; to be most effective as well as commercially valuable.
Methods: All strains investigated in this study isolated from different saline and hypersaline lakes and wetlands from Iran. A total of 65 halotolerant strains were tested in this project. Strains were cultured on a production medium containing 0.3 % uric acid and total salt 3 %. The agar plates were incubated at 30ºC and monitored 48 h. A clear zone indicates the presence of uricase enzyme. Strains with clear zone were selected for quantitative assay with UV visible-spectrophotometer at 293nm. Decrease of absorbance indicated elimination of uric acid by uricase activity.
Results: Our results revealed some species of Halomonas and Paracoccus with high enzyme activity which can be used as a new resource for production of uricase.
Conclusion: This investigation showed that the halotolerant bacteria are potentially resources for uricase under stress condition with a commercially valuable quality and quantities.

Keywords: Uricase, Halotolerant Bacteria, Screening

NATIVE PAGE AS A TOOL FOR SEMI-PURIFICATION OF CATALASE FROM KOCURIA ASB 107

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Background: The aim of this study was the evaluation of possible potential of native polyacrylamide gel electrophoresis as a tool for partial purification of catalase from Kocuria ASB 107.
Methods: The bacterial culture was cultivated in TSB medium and then the biomass was collected within the stationary phase. The pellet was resuspend in 50mM potassium phosphate buffer at pH 7 and washed for 5 times. The cells were lysed after 80min incubation in lysosyme solution at 37°C. The supernatant was isolated by centrifuge and catalase activity of the cell extract was checked by monitoring A240 in the presence of substrate (H2O2). Then the cell lysate was loaded on top of a native polyacrylamide gel (10%). Zymogram was obtained by adding diluted H2O2 on the gel surface. The band of catalase was cut and removed from the gel and check for catalase activity as mentioned above. The remained gel was stained by coomasie blue.
Results: Formation of oxygen bubbles on the gel indicated the precise location of catalase. Bands appeared in the remained gel after staining and destaining process.
Conclusion: According to our result we could observe catalase was partially purified among the mass of proteins in the cell extract as an individual bubble-forming bond on the gel.

Keywords: Catalase, Purification, Native PAGE, Kocuria ASB 107

EVALUATION OF ASSOCIATION BETWEEN CHLAMYDOPHILA PNEUMONIAE AND ATHEROSCLEROTIC PLAQUES IN CORONARY ARTERY DISEASE PATIENTS WITH ABNORMAL ANGIOGRAPHY

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Background: The role of *Chlamydophila pneumonia* in pathogenesis of atherosclerosis is one of the most important discussions in coronary artery diseases.
Methods: In this study, the relationship between Chlamydophila pneumoniae seropositivity and atherosclerotic plaque was evaluated among two groups; one group with significant coronary stenosis and one group with normal coronary angiography. Serum *Chlamydophila pneumonia* IgM and IgG were evaluated and compared in case and control groups.
Results: The IgM and IgG antibodies seropositivity rates were not statistically different between case and control groups, although the rate of positivity was more in case group.
Conclusion: The results of our study could not make a correlation between *Chlamydophila pneumonia* infection and atherosclerotic plaque or coronary artery stenosis neither in acute present nor in past chronic infection. Studies that are more precise are needed to clarify the probable inflammatory cascade that starts with *Chlamydophila pneumonia* infection and ends with development of atherosclerotic plaque.

Keywords: *Chlamydophila pneumonia*, Atherosclerosis, Angiography, Infection

MYCOPLASMA CONTAMINATION IN CELL CULTURES TREATED WITH CIPROFLOXACIN AND ENROFLOXACIN

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Background: In this study, *Mycoplasma*-infected cell lines treatment was done by different dilutions of ciprofloxacin and enrofloxacin that inhibits DNA replication and protein synthesis, treatment and removal of *Mycoplasma* contamination in cell cultures infected verified by PCR method
Methods: Different dilutions of antibiotics such as Ciprofloxacin and Enrofloxacin were used in sequential passages for treatment of the infected Vero cell. Antibiotic treatment with ciprofloxacin and enrofloxacin in contaminated cell to eliminating *Mycoplasma* relying on the lowest passage was compared, the result of removal *Mycoplasma* by antibiotics were determined by PCR method in each step.
Results: Most efficient concentration of Ciprofloxacin and Enrofloxacin was suggested by topsis method, the dilution of Ciprofloxacin and Enrofloxacin and ability of them in removing *Mycoplasma* and also the time of treatment were compared and verified.
Conclusion: Proposed concentration of ciprofloxacin is 60µg/ml, and in the second order it is 30µg/ml,for Enrofloxacin. The best-proposed effective concentrations respectively are 30, 300 and 3µg/ml dilutions. In the present method, treatment with antibiotics was done without any shifting inantibiotic's family.

Keywords: *Mycoplasma*, Cell Culture, Ciprofloxacin, Enrofloxacin, PCR

THE PREVALENCE OF CHLAMYDIA TRACHOMATIS IN PATIENTS WITH BENIGN AND MALIGNANT OVARIAN CANCER BY NESTED PCR METHOD

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Background: The aim of this study was to investigate the prevalence of *C.trachomatis* in patients with ovarian cancer that referred to Imam Hossein Hospital in Tehran, Iran.
Methods: In this descriptive study that was conducted from January 2013 to April 2014, 120 pathological samples were studied which obtained from patients with ovarian cancer who referred to Imam Hossein Hospital. The samples were transferred to the laboratory of Shahid Beheshti University of Medical Sciences. DNA was extracted with QIAamp blood mini kit (Qiagen,USA) according to manufacturer's instructions. In order to detect the presence of C.trachomatis, specific primers for the MOMP (Major Outer Membrane Protein) genes of *C.trachomatis* were designed and evaluated by Nested PCR method.
Results: Out of 120 samples of ovarian cancer,60 (50%) samples were malignant cancer and 60 (50%) were benign cancer as control group. Results of Nested PCR showed that 14(23.3%) malignant samples were positive for the presence of *C. trachomatis*. None of the tissue samples of ovarian benign cancer was positive for *C. trachomatis*.
Conclusion: Our results suggest that the spread of *C.trachomatis* in the female with ovarian cancer may be common. This finding reflects a possible role of *C.trachomatis* in the carcinogenesis of ovarian tumors. *C.trachomatis* infection may play a relative role in the pathogenesis of ovarian carcinomas or it could facilitate its progression.

Keywords: *Chlamydia trachomatis*,Ovarian Cancer, Nested PCR

EVALUATION OF CHLAMYDIA TRACHOMATIS INCIDENCE IN WOMEN WHO HAD ABORTION IN ISFAHAN

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Background: The aim of this study was to evaluate the incidence of CT in miscarriage.
Methods: A questionnaire containing some demographic information and clinical features related to the infection was completed for each woman who had at least one abortion and attended Fertility and Infertility Center in Isfahan. Self-taken vaginal swabs were collected using sterile cotton swap from 84 women.
Results: Out of 84 samples, 14 were excluded due to antibiotic consumption or inadequate swab quality. All the remaining samples (70) were analyzed by plasmid primers. Only one sample (1.43%) showed positivity for CT infection.
Conclusion: The reason for low incidence of CT in these patients could be not only usage of abundant antibiotics but also limitation of sexual partners in comparison to the West. It should be noted that moral, social and religious factors in our society could have resulted in this low chlamydial infection rate among the normal population in contrast to subjects who have multiple sexual partners. However, a larger sample size in future studies will provide data that are more precise.

Keywords: *Chlamydia trachomatis*, Abortion, Iran

DETECTION OF CHLAMYDIA TRACHOMATIS IN FERTILE AND INFERTILE WOMEN IN SANANDAJ BY PCR

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Background: The aim of this study was to determine the prevalence of *C. trachomatis* in infertile and fertile women in Sanandaj (Kurdistan, Iran).
Methods: 104 infertile women referring to Clinic-affiliated Besat hospital in Sanandaj (Kurdistan, Iran) from February to May 2013 were selected as the case group and 104 fertile women with at least one child who referred to non-infertility clinics at the same time were included as the control group. Dacron Swabs were used for sampling from the endocervical canal of women. Cervical swabs were transported to laboratory in 5 mL of Phosphate Buffered Saline (PBS) medium and were frozen at -20℃ until examination. DNA was extracted from samples using DNA extraction kit and subjected to Polymerase chain reaction (PCR) using *C. trachomatis* specific primers. Statistical analysis was performed using the statistical package for the social sciences (SPSS) software version 13.0 for Microsoft windows.
Results: The age ranging for both groups was 14-40 (average age 31.63 years for fertile group and 29.16 years old for infertile patients). In fertile group 6 cases (5.76 %) were positive for *C. trachomatis* while infertile group, *C. trachomatis* was detected from 5 patients (4.80 %).
Conclusion: Prevalence of *C. trachomatis* infection in the two studied groups was almost the same and no significant difference between fertile and infertile groups was observed. According to available resources, in communities where the relative frequency is higher than 4%; screening is recommended. Hence, in order to to reduce the burden of *C. trachomatis*, screening can be considered as a part of the national health programs.

Keywords: *Chlamydia trachomatis*, Prevalence, Infection, Infertility

CHARACTERIZATION OF CHLAMYDIA TRACHOMATIS OMP1 GENOTYPES ISOLATED FROM PATIENTS WITH FOLLICULAR CONJUNCTIVITIS REFERRED TO FARABI HOSPITAL BY RFLP

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Background: In this study, we used Omp1 Genotyping for analysis of samples from patients with follicular conjunctivitis referred to Farabi Hospital.
Methods: Conjunctival scraps (n=90) were obtained from patients who were referred to the diagnostic laboratory of Farabi hospital during 2012. Smears were prepared by rolling half the swab on to the center of a glass slide. Smears were fixed and used for direct immunofluorescence (DIF) method using a genus specific fluorescein isothyocyanate labelled *Chlamydia* monoclonal antibody. In Giemsa stain, diagnosis was based on the prescience of inclusions that were basophilic and stained pinkish –blue. PCR amplification after extraction was performed using CT1 and CT5 primers designed from Omp1 gene. Omp1 Genotyping was performed by RFLP method using AluI.
Results: Among 90 cases examined for *Chlamydia trachomatis* in eyes, 28 patients (31.1%) were positive by DIF and 13 (14.4%) by Giemsa staining and 35(38.8%) showed positive result in PCR. *C. trachomatis* genotypes E (51/2%), G (21/9%), I (14.6%) and F (4.8%) were the most prevalent serovars among patients with follicular conjunctivitis.
Conclusion: In our comparison, PCR detected ocular *C.trachomatis* infection significantly more often than DIF and Giemsa stain. The results of this study show that PCR has higher sensitivity and sensitivity comparing DIF and Giemsa stain. So it is proposed that PCR is used as a conventional method for detecting chlamydial infections in eye. Urogenital serovars were the most prevalent serovars among patients with follicular conjunctivitis.

Keywords: Follicular Conjunctivitis, *Chlamydia trachomatis*, OMP1 (Outer Membrane Protein)

ISOLATION AND GENOTYPING OF CLOSTRIDIUM PERFRINGENS FROM BROILER MEAT IN MASHHAD CITY

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Background: *Clostridium perfringens* is an important cause of bacterial food poisoning worldwide. The disease is caused by *C. perfringens* enterotoxin (CPE) encoded by cpe gene. The aim of this research was to identify the different types of *C. perfringens* and the presence of cpe gene in isolated bacteria from broiler meats presented in retail meat shops of Mashhad city in northeastern of Iran.
Methods: In this study, 200 samples of fresh chicken carcasses were purchased, randomly from different retail meat shops. In laboratory after rinsing the carcasses and culturing in enrichment and selective media, morphological test was performed from suspected colonies. After DNA extraction from the suspected colonies, for confirming the isolates as *C. perfringens*, a PCR assay using specific primers for 16s rRNA gene, and for toxin typing a multiplex PCR assay with specific primers, were performed.
Results: *C.perfringens* was isolated from 31 broiler meat samples (15.5%) and for toxin typing the results showed 9 isolates as type A (29.03%) and 22 isolates as type C (70.96%). In this study, cpe-positive *C. perfringens* were detected in 8 isolates of type C(25%).
Conclusion: Our results indicate that *C. perfringens* type C is the most common type in broiler chicken carcasses.

Keywords: *Clostridium perfringens*، Multiplex PCR، Cpe Gene

IATROGENIC BOTULISM; COMPLICATION OF BOTULINUM TOXIN INJECTION

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Background: Therapeutic use of botulinum toxin type A is usually effective and safe. Iatrogenic botulism emerges with generalized weakness, dysphagia, and respiratory distress and is a rare but significant complication of botulinum toxin type A treatment.
Methods: Reporting a case study.
Results: The patient was a 24-year-old man with chief complaint of bilateral ptosis and progressive weakness of upper and lower extremities, nasal speech, diplopia, dysphagia and dyspnea. In past medical history, there was no canned food consumption, traveling, and contact with ill patient or animal. He did not suffer from any neurologic diseases. The patient has had several injection of Botox (botulin toxin) in the palms due to hyperhyrosis of both hands. The injection was done by dermatologist in 40 points of each hand. He received 1000 units of Botox totally. According to disease course and history of Botox injection iatrogenic botulism was diagnosed. Blood sample was taken for evaluation of botulism. Polyvalent botulinum anti-toxin was started. Gerenaral condition gradually improved. After three weeks he was discharged from hospital without any complication and he could eat, drink and walk.In this case conservative management and anti-toxin provided a good outcome.
Conclusion: Clinicians should be mindful of the risk for systemic botulism when using local injections of the neurotoxin.

Keywords: Botulism, Iatrogenic, Botolinum Toxin

ADHESION SITE OF BOTULINAL TOXIN: IS IT A PROPER VACCINE CANDIDATE?

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Background: The botulinum neurotoxins are the causative agents of botulism and represent a family of seven structurally similar but antigenically distinct serotypes (A to G). The toxins exert their action by blocking the release of the neurotransmitter acetylcholine at the neuromuscular junction. The toxin consists of a 100 kDa heavy chain and 50 kDa light chain held together by a single disulfide bond. The carboxy-terminal (Hc) fragment of botulinum toxin B (BHc) in *E. coli* strain GM2163 was expressed and evaluated in this study.
Methods: All strains of *E. coli* used in this study were primarily cultured on LB broth. The appropriate media were supplemented with ampicillin. DNA fragments which had been generated using the PCR and the synthetic gene was digested cloned into Hind III and Nco I digested plasmid PTG19-T and transformed into *E. coli* strain GM2163. The nucleotide sequence of the cloned fragment was determined to ensure authenticity.
Results: Based on deduced amino acid sequence homology with the Hc fragment of botulinum toxin a DNA fragment (1280 bp) was identified which would encode 428 amino acids at the C-terminus of heavy chain of botulinum toxin B. To enhance gene expression in *E. coli* a gene encoding the BHc was designed to reduce. The complete bhc gene, was created by ligating of the blocks to each other and cloning into plasmid vector in *E. coli.*
Conclusion: It could be suggested to produce a subunit vaccine based on the Hc binding domain of C. botulinum toxin type B which can protect against intra-peritoneal toxin challenge.

Keywords: *Clostridium botulinum*, Bhc Gene, Vaccine

INCIDENCE OF CLOSTRIDIUM PERFRINGENS AND CLOSTRIDIUM DIFFICILE IN PATIENTS WITH GASTROINTESTINAL DISORDERS

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Background: We undertook this study to investigate the presence of *C. perfringenes* in two groups of diarrheal patients (IBD and non- IBD patients).
Methods: A total of 97 stool samples were obtained from patients with gastroenteritis, including 55(5.85%) from non-IBD patients and 39 (41.5%) from IBD patients. The stool samples were examined for the detection of *C. perfringenes* and *C. difficile* after treatment with buffer salin phosphate. The treated samples were cultured on Neomycin Egg Yolk Agar plates supplemented with 5% horse blood and CCFA for isolation of *C. perfringens* and *C. difficile*, respectively and incubated at 37 °C under anaerobic conditions for 2 days. PCR was done on the extracted DNAs of C. difficile and *C. perfringenes* colonies for toxin encoding genes tcdA, tcdB and cpe.
Results: Out of the 97 stool samples tested, *C. perfringenes* and *C. difficile* were detected in 19 (20.2%) and 10 (10.3%) samples, respectively. Two isolates of *C. perfringens* (2.1%) and 10 (10.3%) isolates of *C. difficile* were positive for cpe, tcdA, and tcdB toxin encoding genes. No association was found between type of infection in IBD and non-IBD patients groups.
Conclusion: Overgrowth of toxigenic strains of *C. perfringens* beyond *C. difficile* in diarrheal patients proposes their clinical significance that should be considered by physicians.

Keywords: *Clostridium perfringens, Clostridium difficile*, Gastroenteritis

ENTEROTOXAEMIA CAUSED BY CLOSTRIDIUM PERFRINGENS TYPE A IN GAZELLE

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Background: *Clostridium perfringensis* a gram-positive anaerobic bacterium that is divided into five subtypes. All subtypes produce alpha toxin. In addition, type C produces beta toxin and type D produces epsilon toxin. Type A strains producing only alpha toxin is widespread in the intestines of warm-blooded animals and in the environment. Strains of type A are associated with a wide variety of disease processes in many organ systems of myriad species of domestic animals. The present study describes a natural case of type A enterotoxemia in one gazelle in Kerman.
Methods: A dead, 1-year-old male gazelle was submitted to Razi Research Institute and examined. In necropsy, there was a severe hemorrhage in the serosa of abomasum and small intestine. For bacteriological analysis, a loopful of ingesta from thesmall intestine was immediately inoculated onto blood agar and incubated anaerobically, at 37 ºC. After 48 h of incubation, the colonies with the characteristic double halo of hemolysis were re-streaked onto blood agar medium for purification. After 24 h catalase test and gram staining were done. Culture on egg yolk agar, SIM and lithmus milk under anaerobic conditions confirmsthe presence of *C. perfringensis*. PCR assays have been established to genotype *C. perfringensis* with respect to the genes cpa, cbp, etx, and iap, encoding the alpha, beta, epsilon and iota toxins, respectively. DNA from the thawed suspension was prepared with the Easy DNA kit according to the manufacturer's instructions and the multiplex PCR was done. PCR products were subjected to electrophoresis for 120 min at 80 V. Amplified bands were visualized and photographed under UV illumination.
Results: Based on bacterial culture and toxinotyping of the isolate this case was diagnosed as enterotoxemia by *C. perfringens* type A.
Conclusion: It was concluded from the present study that enterotoxaemia can occur in gazelle with clinicalsigns and pathological lesions similar to those of other small ruminants.

Keywords: *Clostridium perfringens*, Gazelle, PCR

OPTIMIZATION OF REAL TIME PCR FOR DETECTION OF BRUCELL ABORTUS

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Background: Brucellosis is a zoonotic disease transferred from infected animal to human. Six classic species involved *B. abortus, B. melitensis, B. suis, B.canis, B.neotoma* and *B. ovis*. In recent years four new species consist of *B.ceti, B. maris, B. pinipedialis* and *B.inopinata* were reported. Standard and classic methods are time consuming and have low specificity and sensitivity.Several Molcular methods have been developed but many of them are not able to detect all *Brucella* intraspecies biovars, thus developing fast and specific techniques for identification of brucellosis is essential.
Methods: Bases on bioinformatic study, *B. abortus* specific positions were found then a Real time PCR assay was designed. Bacterial samples used in this study consist of *Brucella* standard, vaccinal and 15 filed strains isolated from cow milk. High Pure PCR Template preparation Kit was used for DNA extraction. Sensitivity and specificity assays were performed
Results: Real time PCR was 100% identical with conventional bacteriological method and phage typing assay. Moreover,based on sensitivity testes this presented real time PCR method had detection limit 400 fg *B. abortus* strain 544 template DNA.
Conclusion: Brucellosis detection by classic methods consists of bacterial isolation and serological assays that have sensitivity between 15 to 70 percent. Real time PCR is a fast and sensitive method for detection of brucellosis. In recent years, several studies for detection of *B. abortus* were reported. According to update, GenBank database used primer was not able to cover all *B. abortus* biovars. In this study *B. abortus* specific primers were designed and the study indicate real time PCR and phage typing assay have 100% identical results. In addition, real time PCR has limit of detection 400 fg template DNA, thus we suggest this presented method could be used in *Brucella* epidemiological and surveillance studies.

Keywords: *Brucella*, Detection, Real Time Pcr

MOLECULAR STUDY OF BRUCELLOSIS IN ENDEMIC REGIONS OF Iran BASED ON NOVEL URS-PCR METHOD

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Background: Brucellosis is an infectious disease in human and animals. Bacteriologic-culture-based methods used for *Brucella* diagnosis are hard and time consuming. The aim of this study was to introduce a novel molecular method for detection of two prevalent species of *Brucella* in Iran, *Brucella abortus* and *Brucella melitensis*
Methods: All complete sequences of chromosome 1 with 2.1 Mbp length were compared with all available *Brucella* sequences. A unique loci was found in chromosome one which was capable to differentiate *Brucella abortus* from *Brucella* melitansis. A set of primers was designed in flanking of unique loci. A total number of 136 (88 human and 48 bovine) well-characterised *Brucella* strains have been evaluated and classified by PCR method.
Results: Results of biochemical tests and bacteriophage typing as golden standard indicated that all *Brucella* strains isolated from human and bovine were *B. melitensis* biovar 1 and *B. abortus* biovar 3 respectively. The PCR results were the same as bacterilogical method in detection of the *Brucella* species.
Conclusion: Sensitivity and specificity assay indicated this method is suitable for detection of *Brucella abortus* and *Brucella melitensis*. Note that bacteriological methods are time consuming, need special equipment and conditions for detection of *Brucella* strains, thus we suggest that this novel PCR method, which was designed, based on the specific loci in chromosome 1 could be used for a rapid detection of *Brucella melitensis* and *B.melitensis*. The advantage of this method over other presented methods is that both *B. abortus* and *B.melitensis* is detectable using one-set primer pair simultaneously.

Keywords: *Brucella abortus*; *Brucella melitensis*; Detection; Novel PCR Method

ELECTROCHEMICAL AND BIOLOGICAL TEMPLATE: RAPID TECHNIQUE FOR DETECTION OF ECOLI TG

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Background: The development of rapid and effective tools for the identification and quantitation of *E. coli* is of extreme importance in food analysis, environmental monitoring and clinical diagnostics.
Methods: The biosensor developed in this work is based on electrochemistry reactions. Therefore, the layout of the sensor is patterned as a two-electrode configuration, working electrode (WE) and reference electrode (RE). The RE were prepared by mixing 1.20 g of graphite powder, which had been heated at 700 °C in a muffle furnace for 15 s, with 800 µL of paraffin oil with a mortar and pestle. A WE was prepared in a similar fashion, except that the graphite powder was mixed with a desired weight of bacteriophage. Both RE and WE pastes were packed into a polyethylene tube (2.5 mm diameter), the tip of which had been cut off. Electrical contact to the paste was established via inserting a copper wire thorough flank.
Results: Electrochemical experiments were carried out with an electrochemical interface LCR meter as a signal transducer and an electrochemical cell that contains the two-electrode system. The *E. coli* trapping on bacteriophage was reported by capacitance measurements.
Conclusion: In this work, we have successfully fabricated an electrochemical biosensor with bacteriophage electrodes on a paste substrate. The proposed sensors have good characteristics such as; low detection limit, wide concentration, fast response time and good selectivity coefficient for *Escherichia coli* detection.

Keywords: Bacteriophage, *E. coli*, LCR Meter

ISOLATION, IDENTIFICATION OF XANTHOMONAS STRAIN FROM LEAVES OF LEMON TREES AND STUDYING THE PRODUCTION OF XANTHAN GUM

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Background: Xanthan gum is an important microbial polysaccharide, which is synthesized by bacteria of the genus *Xanthomonas* and considered as pest for a lot of plants. This research has been done with the purpose of isolating and identifying *Xanthomonas* strain from leaves of lemon trees and the production of Xanthan gum.
Methods: Sampling from marked leaves of lemon trees that were infected by bacterial canker was performed and isolating the disease agent on culture environment of YDC was carried out at 28 °C temperature after 24-48 hours. Then, the obtained colony was assessed for morphological and biochemical characteristics viewpoints. An environment for producing Xanthan gum was used to produce the gum and then final identification of separated bacteria was studied by 16S rRNA method.
Results: In this research, a new strain under the name of *Xanthomonas campestris* strain saba.ton was known. This bacteria is gram negative, obligate aerobic and short *Bacillus* and also its colony on agar medium, is yellow, mucoide and viscous and also the amount of Xanthan production was reported to be 0.081 -1.5g / 100 ml.
Conclusion: In the present study, the identification andthe ability of a local strain in production of Xanthan gum have been studied.Without optimizing the growth conditions it had a potential power in the production of Xanthan gum and purification of the produced Xanthan from the isolated strain makes it usable for food consumptions and also in other cases.

Keywords: *Xanthomonas*, Xanthan Gum, Lime.

ESCHERICHIA COLI AND SHIGELLA GENUS DETECTION IN WATER SAMPLE BY PENTAPLEX PCR

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Background: The aim of this study was to determine the most ubiquitous *Escherichia coli* specific genes to apply as a genetic marker to speed up the detection time and the sensitivity and introduce a multiplex PCR to detect and screen this marker organism in water sample as soon as possible. Since *Shigella* species share great sequence similarity, so we included a specific gene corresponding to putative integrase enzyme to differentiate *Shigella* species from *Escherichia coli* .
Methods: Out of 1956 water samples, one hundred and six *Escherichia coli* strains, isolated from different water sources of Tehran province (Jalalieh water treatment center, kan water treatment center, number 3, 4 and 5 water treatment centers) within a 1 year were characterized by standard methods based on fast lactose fermentation and IMViC tests. All the isolates were kept in glycerol at -70ºC until the final procedure.We extracted DNA from all isolates applying Accuprep Bioneer column kit. New specific sets of primers including structural genes like uspA, phoE and enzyme coding ones such as gadA/B, uidA for *Escherichia coli* and put int for *Shigella* were designed in a novel pentaplex PCR by Allele ID 7.6 (Table 1). Following checking the specificity of any set of primers in NCBI, dimer formation and primers interaction, applying Oligo analyzer 1.03, we optimized the multiplex at different temperatures and DNA concentration gradients.
Results: Among 978 water samples all tested for *E. coli*, we characterized 106 strains by standard methods such as lactose fermentation and IMViC test. Following pentaplex PCR development and optimization, we looked for the uspA, phoE, gadA/B, uidA for *Escherichia coli* and put int for *Shigella* genus. While detecting all 4 genes of *E. coli*, our results showed this multiplex PCR is a great tool to detect such organism in water samples within a short period of time. Our results showed that all 106 strains carried the above-mentioned genes which confirms and verifies the accuracy of our approach.
Conclusion: Our results showed the ubiquitous specific genes of *Escherichia coli,* as well as the other cells, are the structural ones encoding 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 the selection pressure. Besides the structural ones, the above mentioned genes encoding the enzymes (gadA/B, uidA) are also prevalent because they belong to essential or housekeeping genes.

Keywords: *Escherichia coli, Shigella*, Pentaplex PCR, Water Sample

DIRECT IDENTIFICATION OF STREPTOCOCCUS AGALACTIAE AT VAGINAL COLONIZATION IN PREGNANT WOMEN BY PCR

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Background: *Streptococcus agalactiae* is a commensal organism but it may cause infection in susceptible hosts including newborns, pregnant or postpartum women. Applying rapid, accurate, and sensitive method for detecting GBS and receiving intrapartum antibiotic prophylaxis (IAP) at delivery have been demonstrated to increase treatment possibility of carrier pregnant women and decrease the rates of GBS vertical transmission to infants.The aim of this study was to evaluate PCR assay targeting 16S rRNA primers compared with conventional culture method for direct detection of GBS in vaginal specimens of pregnant women at 35–37 weeks of gestation in Hamadan.
Methods: 203 vaginal specimens of pregnant women at 35–37 weeks of pregnancy from June 2013- February 2014 were evaluated for detection of GBS using culture method and PCR assay.
Results: The prevalence of GBS in 203 collected samples was 7.39% using culture method and 19.70% using PCR assay. 25 specimens were determined to be positive by PCR and negative by culture; 2 specimens were positive by culture and negative by PCR. Generally, a total of 42 specimens (20.69%) were considered true positive. PCR results in comparison to culture (as gold standard) revealed sensitivity of 88.24%, specificity of 87.44%, positive and negative predictive value of 35.71%, 98.95%, respectively, and accuracy of 87.50%.
Conclusion: The study data demonstrated that performing only culture method leads to missed false negative carrier individuals. Thus, it is recommended that both the PCR assay and conventional culture method to be performed routinely in order to detect GBS in pregnant women accurately. Besides, PCR diagnosis demonstrated a shorter turnaround time when compared with time consuming culture method.

Keywords: *Streptococcus agalactiae*; Pregnancy; Polymerase Chain Reaction; Culture

EARLY DETECTION OF SALMONELLA ENTERICA SEROTYPE INFANTIS

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Background: Salmonellosis is one of the most common food-induced diseases which is widely distributed all over the world and is known as one of the most serious public health concerns. Therefore, it is necessary to obtain developed methods in order to achieve a quick response in identifying *Salmonella* bacterium. This study aimed to evaluate hisD genes as a potential tool for early recognition of *Salmonella enterica* serotype Infantis.
Methods: Examined isolates in this study were separated from patients with *Salmonella enterica* serotype Infantis infection in several hospitals of Tehran and were prepared for polymerase chain reaction following bacteriological and biological tests. Afterward, a specific pair of primer was designed for hisD gene amplification by means of related software. Following isolates` genome extraction, PCR was done in order to identify *Salmonella enterica* serotype Infantis.
Results: Following the experiments above, the results of PCR product electrophoresis revealed a 651bp bond for hisD gene and primers didn't make a bond with enterobacteriacea strains such as *Shigella* and E.coli.
Conclusion: According to the results, hisD gene has the ability to recognize and identify *Salmonella enterica* serotype Infantis strains and it is also able to differentiate it from the rest of enterobacteriacea strains.

Keywords: *Salmonella infantis*, Hisd Gene, PCR

COMPARISON OF FOUR DIAGNOSTIC METHODS FOR DETECTION OF METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS

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Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) is a well-known pathogen with a worldwide distribution. Due to the increasing rate of MRSA infections, implementing of reliable, accurate and rapid testing for diagnosis of MRSA is necessary. The aim of this study was to compare four diagnostic methods for detection of MRSA isolates.
Methods: From December 2012 to April 2014, we collected 120 *S. aureus* isolates from three hospitals affiliated with Tehran University of Medical Sciences. MRSA isolates were detected by four different methods including cefoxitin disc diffusion test, oxacillin disc diffusion test, minimum inhibitory concentration (MIC) of oxacillin as determined by MIC test strip, and mecA detection by PCR.
Results: Out of 120 *S. aureus* isolates, cefoxitin disc diffusion test, oxacillin disc diffusion test and MIC test strip identified 60 (50%), 48 (40%), 55 (45.83%) isolates as MRSA, respectively. The sensitivity and specificity for oxacillin disc diffusion, cefoxitin disc diffusion and MIC of oxacillin were 80% and 100%, 100% and 100%, and 91.6% and 100%, respectively.
Conclusion: Cefoxitin disc diffusion test is a reliable substitute for detection of MRSA in clinical laboratory where MIC detection and molecular methods are not accessible.

Keywords: *Staphylococcus aureus*, Methicillin Resistance, Microbial Sensitivity Tests

DESIGN OF NOVEL INTERNAL POSITIVE CONTROL (IPC) FOR MOLECULAR DETECTION OF INFECTIOUS BRONCHITIS VIRUS (IBV) THROUGH REAL TIME RT PCR.

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Background: The aim of the present survey is to design and construct a plasmid containing recombinant 5'UTR and 3'UTR genes as positive control and internal positive control.
Methods: We constructed an IPC that can be amplified by the same primer pair as wild-type target RNA and the different site for probe. IPC was obtained by insertion of an exogenous DNA fragment into reference strain target by recombinant PCR and finally the production was colonized in pTZ57RT.Afterwards, the plasmid constructs were transformed into *E. coli* TOP10F´ host strain. Screening the desired recombinant clone was carried out using colony PCR
Results: Sequencing confirmed the presence of the desire insert (IPC sequence) in recombinant plasmid. Consequently, the IPC fragment was longer than the target gene while both ends had similar attachments to the same primer pair. The probe site is different in positive control and IPC.
Conclusion: An internal control of amplification was constructed by recombinant PCR to detect PCR inhibitors. This exogenous DNA was included in the reaction mixture and amplified with the target gene. This detection was successfully applied to the diagnosis of Infectious bronchitis (IB)in clinical samples. The designed IPC assay has proven to be an effective tool for monitoring inhibitors of RT_-PCR and builds confidence in negative results obtained with agent specific assays.

Keywords: Infectious Bronchitis, Iran

DEVELOPMENT AND CLINICAL VALIDATION OF LOOP-MEDIATED ISOTHERMAL AMPLIFICATION METHOD FOR RAPID DETECTION OF BORDETELLA PERTUSSIS BASED ON INSERTION SEQUENCE IS481

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Background: In spite of the existence of conventional culture method and developing PCR-based assays, yet the accurate detection of *Bordetella pertussis* is a problem. In this study, we developed a Loop-Mediated Isothermal Amplification (LAMP) assay based on IS481 and it has evaluated the efficiency, rapidity, sensitivity, and specificity of IS481 LAMP in comparison with real-time PCR with the same target for the detection of *B. pertussis* clinical specimens.
Methods: One hundred and sixty-five nasopharyngeal swabs were used to be assessed by LAMP and real-time PCR. Amplified LAMP products were detected by adding SYBR® Safe DNA Gel Stain. Illumination under UV light and the green color for positive samples were visible by unarmed eyes.
Results: The sensitivity and specificity of the LAMP assay were reasonable in comparison with real-time PCR. In this study, they were 87.5% and 92.9% for IS481 target, respectively. Amplification of 10 fg by IS481-LAMP showed the sensitivity of 2.26 genomic copies of *B. pertussis*.
Conclusion: The developed LAMP assay based on IS481 target helps rapid and sensitive detection of *B. pertussis*, and it is cheaper than previous conventional real-time PCR. This IS-481 LAMP assay has the merit of being used as a diagnostic method for *B. pertussis*.

Keywords: LAMP, *Bordetella pertussis*, IS481

DEVELOPMENT OF ENZYME-LINKED IMMUNOSORBENT ASSAY WITH A NUCLEOPROTEIN AS AN ANTIGEN FOR DETECTION OF ANTIBODIES AGAINST AVIAN INFLUENZA VIRUS

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Background: The aim of this study was to produce recombinant nucleoprotein (NP) and development of an ELISA test with it.
Methods: For this purpose, the coding region of nucleoprotein (NP) gene of A/chicken/Iran/AH-1/06(H9N2) AI isolate was amplified by RT-PCR and cloned into a prokaryotic expression vector (pMal-C2x) and transformed into *E. coli* BL21(DE3). The recombinant protein MBP-NP was expressed in *E. coli* and was purified using amylase resin chromatography column and used as the diagnostic antigen to develop a NP-based type specific indirect enzyme linked immunosorbent assay (ELISA) for detecting antibodies to AI for chicken sera. The NP-ELISA was compared with a commercial AIV ELISA kit (IDEXX- USA).
Results: Excellent agreement was obtained between NP-ELISA and commercial ELISA kit (k= 0.932). Results indicated that in house NP- ELISA is more sensitive than commercial ELISA for detecting the antibodies in chicken inflected with AIV but the difference was not significant.
Conclusion: This finding indicates that the indirect ELISA based on rNP developed in our laboratory is a sensitive and specific test and can be used for diagnosis and seroepidemiological investigation in long-term AIV program prevention and control.

Keywords: Avian Influenza Virus, Nucleoprotein, H9N2, NP-ELISA

A SURVEY ON OROPHARYNGEAL COLONIZATION BY NEISSERIA LACTAMICA, OTHER NON-PATHOGENIC NEISSERIA SPECIES AND MORAXELLA CATARRHALIS IN HEALTHY YOUNG CHILDREN IN AHVAZ, Iran

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Background: *Neisseria lactamica* as a main commensal in oropharynx during the childhood is related to the induction of a natural immunity against meningococcal meningitis. Also *Moraxella catarrhalis* in oropharynx of children is a predisposing factor for otitis media infection. This study was aimed to investigate the frequency of the *Neisseria lactamica*, other non-pathogenic *Neisseria* spp. and *M. catarrhalis* in the oropharynx of healthy young children in Ahvaz, Iran by the two phenotypic tests and Polymerase chain reaction (PCR).
Methods: A total of 192 oropharyngeal swab samples of the healthy young children were studied during four months. Swabs were plated onto enriched selective media and non-selective media. Gram-negative, oxidase-positive diplococcic isolates were identified by several conventional biochemical tests. The PCR and sequencing were used for confirming the accuracy of laboratory diagnosis in identifying *N. lactamica* and *M. catarrhalis*.
Results: Totally we identified 192 healthy young children with the mean age of 5.93±2.5903: *N. lactamica* (21.9%) in the aged 1-9 years; *N. mucosa* (6.3%); *N. sicca* (7.8%); *N. cinerea* (1.6%); *N. subflava* (biovar subflava) (4.2%); *N. subflava (biovar perflava)* (28.1%); *N. subflava* (biovar flava) (7.3%) and *M. catarrhalis* (42.7%).
Conclusion: It was the first screening report of the healthy young children by colonization of *N. lactamica* and other nonpathogenic *Neisseria* spp. in oropharynx in Ahvaz, Iran.

Keywords: *Neisseria lactamica*; *Moraxella catarrhalis*; Colonization; Children

EVALUATION OF THE BACTERIA RECOVERED FROM VAGINAL SWABS IN WOMEN OF PARS HOSPITAL IN TEHRAN, Iran

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Background: The isolation of vaginal bacteria from females indicates colonization or infection and their complexity are influenced by sexual activity, childbirth, tampon use and other happenings during their reproductive life. The objective of this study was to do the statistical analysis for the recovered bacteria from vaginal swabs of infected women in Pars hospital, Tehran, Iran.
Methods: Vaginal swabs of patients admitted to Pars hospital during the March 2013 to June 2013 were cultured and antimicrobial susceptibility of isolated bacteria was determined by disk diffusion methods. Thereafter, all the data collected from these isolates were analyzed statistically using SPSS software.
Results: From 50 bacterial positive vaginal swabs, 49 of them were recovered from outpatients and only one was from hospitalized patient. Most of isolates (34 cases, 68%) recovered from female in the age group of 15-44 years. The most prevalent isolated bacteria from vagina included Group B Streptococci (GBS) (34%), *E. coli* (32%), *Enterococci* spp.(18%) and *Klebsiella* spp. (6%), respectively. All GBS isolates showed sensitivity to penicillin, ampicillin, vancomycin and cefotaxime. Resistance to erythromycin and clindamycin was shown in 47.1% and to cefepime and levofloxacin in 15.4% and 17.6% of GBS isolates, respectively.
Conclusion: Although GBS are colonized in the vagina and almost 10-30% of pregnant women possess these bacteria in their vagina, it has been shown that 50-70% of them may transfer GBS to their neonates. Therefore, in order to prevent neonatal GBS infection, isolation of GBS from women in reproductive age is important. The finding indicated that the most recovered bacteria from vagina of studied patients were GBS and from the women at the age group of 15-44 years, it is important to use proper methods for diagnosis of these infections especially in pregnant women to prevent newborn infections. Fortunately, the frequency of resistance to common antibiotics is low in GBS isolates.

Keywords: Vaginal Swabs, Group B Streptococci (GBS), Antimicrobial Susceptibility

RAPID DETECTION OF EXTENDED SPECTRUM B-LACTAMASES (ESBLS) PRODUCING ISOLATES OF KLEBSIELLA PNEUMONIA BY A NEW COLORIMETRIC MEDIUM

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Background: The purpose of this study was to evaluate a new rapid colorimetric method for detection of ESBL producing *Klebsiella pneumoniae*. This method is capable to detect ESBL producing bacteria, by a color change in the colorimetric medium within 5 to 6 hours, which is due to metabolic activity of growing bacteria. The method is based on CLSI standard disk diffusion method performed on the colorimetric medium
Methods: Fifty-four clinical isolates of *Klebsiella pneumoniae* obtained from Pasteur Institute of Iran and Iran University of Medical science were used for evaluation of this medium. 25 ESBL positive and 29 ESBL isolates were compared by CLSI disk diffusion ESBL (phenotypic confirmatory test) criteria by Mueller Hinton and colorimetric medium. The tested antibiotics included ceftazidim (CAZ), ceftazidime + clavulanate (CZA), cefotaxime (CTX), cefotaxime +clavulanate (CTC).
Results: A color change was observed for ESBL producing bacteria within 5 to 6 hours by the colorimetric medium. These results were in line with the results of overnight incubation on Mueller Hinton agar.
Conclusion: This new colorimetric medium can be used for rapid and reliable detection of ESBL producing *Klebsiella pneumoniae* within 5 to 6 hours

Keywords: Colorimetric Medium, Rapid Detections, CLSI Disk Diffusion Test

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MOLECULAR STUDY OF ERYTHROMYCIN RESISTANCE FACTORS IN GRAM POSITIVE COCCI ISOLATED FROM IN SHAHID RAJAEE TEACHING HOSPITAL, QAZVIN

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Background: Gram positive cocci organisms show many molecular important resistance factors to erythromycin which would be important nosocomial infections agents. This study was conducted to determine the molecular factors for resistance to erythromycin, in gram positive cocci isolated from laryngoscope in Shahid Rajaee teaching hospital, Qazvin.
Methods: This molecular descriptive study was carried on the samples collected from the laryngoscope in 1392. In aseptic conditions; samples were transferred to microbiology laboratory. Bacterial identification was performed usingthe standard laboratory methods. Antibiotic sensitivity was carried out using usual disk diffusion agar method according to CLSIrecommendation. PCR assay was performed and and the products of it was sequenced to check the presence of mef, msrA, erm A, erm B, ermC genes.
Results: 21 isolates (41/1%) out of 51 were resistance to erythromycin. In Coagulase –ve Staphylococci (CONS) were positive for ermC (4-19.5%)and mef (1-4.76%), respectivily. Alpha hemolytic Streptococci were also positive for both ermA and ermB (2-9.52%) and gamma hemolytic Streptococci carried ermB (4-19.5%), ermA and mef (1-4.76%).
Conclusion: The most important finding in this study is the presence of the erythromycin resistance genes for the mef, msrA, ermA, ermB, erm C in collected isolates from laryngoscope in our hospital.Since laryngoscope can potentially be carried in resistant isolates, disinfection and control the infection is essential before using this device in patients.

Keywords: Laryngoscope, Gram Positive Cocci, Erythromycin, Resistant Genes

PLASMID-MEDIATED QUINOLONE RESISTANCE DETERMINANTS QNR AND AAC(6')-IB-CR IN FLUOROQUINOLONE-RESISTANT ENTEROBACTERIACEAE IN HAMADAN, Iran

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Background: The quinolone group is an important class of broad-spectrum antimicrobial agents. Plasmid mediated quinolone resistance (PMQR) determinants have emerged as a significant concern in recent years. This study reports the screening of resistant-isolates to Fluoroquinolone antimicrobial agents for PMQR determinants and detection of Qnr and aac(6')-Ib-cr genes.
Methods: Total of 100 fluoroquinolone-resistant Enterobacteriaceae were isolated from 3 hospitals in Hamadan from October 2012 to June 2013. The isolates were identified by biochemical tests and confirmed by PCR. Antimicrobial susceptibility to 14 antimicrobial agents including levofloxacin and ciprofloxacin was determined by Clinical Laboratory Standards Institute (CLSI).Disk diffusion methods and ciprofloxacin MIC were obtained by broth microdilution. Then the isolates were screened for the presence of qnrA, qnrB, qnrS and aac(6')-Ib-cr genes using PCR assay.
Results: Among the screened isolates, 64 strains (64%) of *Escherichia coli,* 23 strains (23%) of *Klebsiella pneumoniae*, 13 strains (13%) of Proteus mirabilis were collected as quinolone-resistant isolates. Out of 100 isolates, two (2%) isolates were positive for qnrS, seventeen (17%) isolates were positive for qnrB and we did not find the qnrA gene in any of the isolates. There were also 32 positive isolates for aac(6?)-Ib-cr gene.
Conclusion: In this study we describe the prevalence of qnr and aac(6')-Ib-cr genes in Fluoroquinolone-resistant Enterobacteriaceae in Hamadan. The carriage rate of multidrug-resistant Enterobacteriaceae in healthy people in Hamadan City is extremely high. Moreover, genes encoding transferable quinolones, in particular aac(6')-Ib-cr, are highly prevalent in these strains.

Keywords: Enterobacteriaceae, Antibiotic Resistance, Fluoroquinolone.

IN VITRO INHIBITORY ACTIVITY OF ODONTOBUTHUS DORIAE SCORPION VENOM AGAINST STAPHYLOCOCCUS AUREUS

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Background: Microbial antibiotic resistance is a challenging medical problem nowadays. Then, it is of considerable interest to develop antibiotics with a new mechanism of action, which can potentially evade the emergence of drug resistance. In the search for such new agents, we are looking for effective antibacterial natural venoms that have potential activity against *Staphylococcus aureus* and *Escherichia coli*
Methods: In our investigation, we studied the antibacterial effects of Odontobuthus doriae scorpion venom against *Staphylococcus aureus* and *Escherichia coli* bacterial strains. Several concentrations (1, 5, 10, 20, 40 and 80 µg/ml) of crude venom were tested for antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* by disc-diffusion susceptibility at different time intervals.
Results: All of the concentrations of crude venom have significant antibacterial effects against *Staphylococcus aureus* after 48 h of incubation; while *Odontobuthus doriae* concentrations have no antibacterial effects on *Escherichia coli* .
Conclusion: In conclusion, our findings demonstrated *Odontobuthus doriae* scorpion venom has an antibacterial activity against *Staphylococcus aureus* and could be used as a new drug for disease induced by *Staphylococcus aureus*.

Keywords: Antibacterial Activity, *Staphylococcus aureus*, *Escherichia coli* .

PREVALENCE AND ANTIBIOTIC RESISTANCE PATTERNS IN BACTERIA ISOLATED FROM URINE IN QAZVIN BU-ALI HOSPITAL FROM 2012 TO 2013

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Background: This study aimed to identify the prevalence of bacterial etiologic agents isolated from the urine culture and to investigate the antibiotics susceptibility of bacteria in one year at Bu-Ali hospital in Qazvin.
Methods: In this cross-sectional study, 7200 urine samples were isolated and antimicrobial susceptibility testing differential disk diffusion method was performed according to the National Committee for Clinical Laboratory Standards (NCCLS). Data analysis was carried out using SPSS software ver.16.
Results: The most common bacteria from urine samples were *Escherichia coli, Klebsiella pneumonia*, *Enterococcus faecalis*. Based on the results, ciprofloxacin is the most effective antibiotic.
Conclusion: The current study showed that the resistance of common bacteria in urine samples has increasedto antibiotics used to treat urine infections. Ciprofloxacin is the effective antibiotic in treatment of urinary tract infections.

Keywords: Bacterial Resistance, Urine Culture, Antibiogram

THE PREVALENCE OF MULTIDRUG-RESISTANT ESCHERICHIA *COLI* AMONG PATIENTS WITH URINARY TRACT INFECTION REFERRED TO IMAM REZA HOSPITAL OF URMIA

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Background: The purpose of this study is to investigate the prevalence of multidrug- resistance in ceftazidime-resistant *Escherichia coli* isolated from patients with urinary tract infection (UTI),that referred to Imam hospital of Urmia in the first half of 1392.
Methods: In this cross-sectional study, all urine samples of patients admitted to Urmia ImamHospital in the first half of 1392 were enrolled. After being cultured in EMB(Eosin methylene blue) and blood agar medium then differential medium and diagnose strains, resistant strains to ceftazidime That antibiogram pattern of them with disk diffusion method with antibiotics: cefepime, ceftazidime, cefotaxime amikacin, Nitrofurantoin, gentamicin, trimethoprim, nalidixic acid, ceftriaxone, imipenem, cefotaxime, Cefixime, cephalexin, ciprofloxacin, and aztreonam, cotrimoxazol, cefepime using standard National Committee for clinical laboratory (CLSI) were studied.
Results: Total 8560 urine samples were examined, of which 1260 (14.7%) were positive cultures(UTI). 812 (64.5%)women and 448 (35.5%)men were from positive cultures that 902 samples from positive cultures were Ecoli of which 181 samples showed resistance to ceftazidime.Patterns of antibiotic resistance were respectively: Cefixime, Ceftriaxone, cephalexin (100%), Aztreonam (96.8%), Cefepime (95.2%), Nalidixic acid (87.3%), trimethoprim sulfamethoxazole (66.6%), ciprofloxacin (63.4%), gentamicin (53.9%), nitrofurantoin (23%), amikacin (14.2%). All the isolates were susceptible to imipenem.
Conclusion: In this study, the most resistance was observed to antibiotics: cefexime,ceftriaxone, cephalexin, aztreonam, cefepime, Nalidixic acid. So we recommend doctors of this city not to use these antibiotics for treatment,especially for treating urinary tract infections.

Keywords: Antibiotic Resistance, *E. coli*, Mdr, Urmia

ANTIMICROBIAL RESISTANCE IN CAMPYLO-BACTER

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Background: Most *Campylobacter* infections are self-limiting but antimicrobial treatment (macrolides, fluoroquinolones) is necessary in severe cases. Rapidly evolving next-generation sequencing technologies may offer beneficial instrument in the future for the prediction of resistance in *Campylobacter*.
Methods: Pulsed-field gel electrophoresis (PFGE) has been widely used for molecular typing of *C. jejuni* because of its high discriminatory power. The PCR assay could identify *Campylobacter*-contaminated samples that were negative using the cultural method, agar dilution and broth microdilution, while a disk diffusion method was recently standardized by the European Committee on Antimicrobial Susceptibility Testing.
Results: Pulsed-field gel electrophoresis (PFGE) method showed that there were several points of cross-contamination of product along the chain, and a high diversity of PFGE types with antimicrobial resistance to ciprofloxacin and tetracycline in the retail products. in PCR method we find susceptibility test results showed that 98.4% of isolates were resistant to one or more antimicrobial agents.in agar dilution and broth microdilution method High levels of resistance to tetracycline and ciprofloxacin in humans, retail meats, and food animals are reported, but resistance to erythromycin and gentamicin in *C. jejuni*, which causes the vast majority of *Campylobacter* infections.
Conclusion: However, great strides have been made in the past decade in standardizing in vitro susceptibility testing methods for *Campylobacter*, the variety of methods and commentary criteria used reflects the need for further harmonization *Campylobacter*

Keywords: *Campylobacter*, Pulsed-Field Gel, PCR

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EPIDEMIOLOGY OF AZOLE RESISTANT ASPERGILLUS FUMIGATUS AND ASPERGILLUS FLAVUS IN Iran

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Background: *Aspergillus flavus* and *A. fumigatus* are the leading causes of invasive and non-invasive aspergillosis. The occurrence of *A. flavus* is worldwide especially in tropical and subtropical regions. The vital importance of *A. flavus* has increased in the last years. Presently the emergence of resistance to antifungal agents among Aspergillus species is dramatically increasing. Therefore, in the present study, we evaluated the in vitro activity of five antifungal agents against *A. flavus* and *A. fumigatus* isolated from different sources.
Methods: In total, 100 strains of *A. flavus, which* have been isolated from various specimens (nail, bronchoalveolar lavage, paranasal sinus) from suspected patients of aspergillosis patients. All strains were identified based on conventional methods and subsequently confirmed by DNA sequencing of the β-tubulin gene. The minimal inhibitory concentrations (MIC) of Amphotericin B, itraconazole, voriconazole, posaconazole and minimal effective concentrations (MEC) for caspofungin were determined using the broth microdilution method in accordance with the guidelines of Clinical and Laboratory Standards Institute (CLSI) document M38-A2.
Results: The resulting MIC90 s for *A. flavus* strains showed an increasing order, as follows: Caspofungin (0.031 µg/ml), posaconazole (0.25 µg/ml); voriconazole (0.5 µg/ml), itraconazole (1 µg/ml) and amphotericin B (4 µg/ml). Although MIC90 for *A. fumigatus* strains was different as follows: Caspofungin (0.016 µg/ml), posaconazole (0.125 µg/ml); voriconazole (0.5 µg/ml), itraconazole (0.5 µg/ml) and amphotericin B (2 µg/ml).
Conclusion: The present study based on in vitro activity showed that posaconazole followed by caspofungin, voriconazole might have a potent activity, and it could be the best alternative for previous antifungal

Keywords: Antifungal Susceptibility, *A. Flavus, A. Fumigatus*

PREVALENCE OF ANTIMICROBIAL RESISTANCE IN ESCHERICHIA COLI ISOLATED FROM FECAL SAMPLES OF SHEEP SLAUGHTERED AT KERMANSHAH ABATTOIR

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Background: Because of the rapid development and spread of antimicrobial resistance, it is important to monitor antimicrobial resistance in pathogenic zoonotic and commensal bacteria of animal sources. In this study, the prevalence of antimicrobial resistance among commensal *Escherichia coli* isolated from fecal samples of sheep slaughtered at Kermanshah abattoir was investigated.
Methods: 100 fecal samples were collected form sheep slaughtered at Kermanshah abattoir during summer and fall seasons. Samples were cultured on EMB agar and obtained colonies were confirmed as *Escherichia coli* based on the biochemical tests results. Totally 85 isolates were obtained out of 100 fecal samples. Isolates were tested for antimicrobial agent susceptibility to 10 antibiotics (colistin, gentamicin, tetracycline, trimethoprim-sulfamethoxazole, amoxicillin-clavunic acid, enrofloxacin, ampicillin, cephotaxime, neomycin and florfenicol), using disc diffusion method.
Results: 92/94% (n=79) of isolates were resistant to at least one of the antimicrobials. 64/7% (n=55) of isolated *E. coli* were multidrug resistant and the highest resistance rate was detected for Tetracycline (76/5%) and Ampicillin (75/3%). No resistance was detected against Gentamicin and florfenicol.
Conclusion: In conclusion, this study demonstrates that the prevalence of antimicrobial resistance to some of the antimicrobials is high among *E. coli* isolated from fecal samples of sheep slaughtered at Kermanshah abattoir (west of Iran) and this can be a crucial issue for both of human and livestock health.

Keywords: Antimicrobial Resistance, *Escherichia coli,* Sheep, Fecal Samples

FREQUENCY OF MUTATIONS ASSOCIATED WITH RIFAMPICIN RESISTANCE IN MYCOBACTERIUM TUBERCULOSIS STRAINS ISOLATED FROM PATIENTS FROM WEST OF Iran

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Background: Tuberculosis (TB) is a devastating infectious disease causing high mortality and morbidity worldwide. The most serious threat related to tuberculosis control is the recent emergence of drug-resistant tuberculosis strains. The aim of the present study was to identify various types of mutations in rpo B region from rifampicin resistant strains isolated from sputum of tuberculosis patients.
Methods: Drug susceptibility testing of 125 *Mycobacterium tuberculosis* isolates was determined using the CDC standard conventional proportional method. Target DNA of *M. tuberculosis* was amplified by PCR, hybridized and scanned. We used the LCD array to detect mutations within the 90 bp rpoB region. Each array is a transparent, pre-structured polymer supports using a non-fluorescent detection principle based on the precipitation of a clearly visible dark substrate.
Results: Of the 125 *M. tuberculosis* isolates, 35 (28%) were found to be rifampicin resistant and using the LCD array revealed point mutations at 9 different codons as follows S512T(AGC→ACC) (20%) D516V (GAC→GTC)(20%) H 526D (CAC→GAC) (6%)H526R(CAC→CGC)(20%) H526Y (CAC→TAC) (23%) S531W (TCG→TGG)(8%). The most frequent site mutations were L511P (CCG→ CTG) (46 %) followed S531l (TCG→TTG) (40%) and D516Y (GAC →TAC) (26%).
Conclusion: Phenotypic testing is time-consuming and needs laboratory facilities. Therefore, there is a need for rapid molecular methods for detection of mutation in drug resistance. Microarray rpoB can be used to detect rifampicin resistance determining region (RRDR) associated site mutations of rifampicin resistant *M. tuberculosis* isolates.

Keywords: *Mycobacterium tuberculosis*; Rifampicin Resistance; Rpob Gene

E-TEST ANTIBIOTIC SUSCEPTIBILITY OF STAPHYLOCOCCUS AUREUS STRAINS ISOLATED FROM HOSPITAL ACQUIRED INFECTIONS OF IMAM KHOMEINI HOSPITAL, ILAM, Iran

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Background: *Staphylococcus aureus* is one of the most important pathogens of human beings all over the world and in all ages. The epidemiology and resistant pattern of this bacterium is not clear in Ilam hospitals, capital of Ilam province, Iran.
Methods: The antimicrobial patterns of *S.aureus* strains isolated from health-care –associated infections of different hospitalization wards including surgery and ICU have been investigated at Ilam Hospitals. Bacteria isolated from different samples in the microbiology laboratory of Ilam University of Medical sciences strains were identified under standard techniques.
Results: Antibiotic susceptibility and Minimum Inhibitory Concentrations was done by E-test and routine antibiotics. A total of 30 *S.aureus* isolates were isolated from urinary (12; 40%), wound (10; 33.3%), lung (5; 16.6%), and burn (3; 10%) infections. All of the isolates were susceptible to vancomycin and linezolide and 10% of strains were methicillin resistant. Resistance percent for other drugs were as follow: piperacillin /tazobactam 4(13.3%), ceftriaxone 11(36.3%), amoxicillin 13(43.4%), amikacin (3.3%), ceftazidime 2(6.6%), tobramycin 3(9.9%), tetracycline 11(36.3%), gentamicin 3(9.9%). Five isolates had MICs≥1.5 µg/ml against vancomycin.
Conclusion: Although about 10% of our isolates were resistant to methicillin, and 100% were susceptible to vancomycin and linezolide, but in some of the isolates we encountered a rise in MICs against vancomycin. This point emphasizes the implementation of related control measures in hospitals for further control of the treatment of resistant infections.

Keywords: *Staphylococcus Aureus*, Antimicrobial Susceptibility, MRSA, Health-Care-Associated Infections.

RESISTANCE TO TETRACYCLINE AND VANCOMYCIN OF STAPHYLOCOCCUS AUREUS ISOLATES FROM SANANDAJIAN PATIENTS

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Background: The increase of *Staphylococcus aureus* resistance to antibacterial drugs is one of the major health concerns, therefore studying antibiotic resistance of *Staphylococcus aureus* is very important and it has a main role in preventing the emergence of resistant strains. This study was done to determine the prevalence and genetic basis of tetracycline and vancomycin resistance in *Staphylococcus aureus* in Sanandaj city.
Methods: One hundred and fifty clinical isolates of *S. aureus* were collected from Sanandaj Hospital. Susceptibility to antibiotics (tetracycline and vancomycin) was determined by disk agar diffusion method with minimal inhibitory concentration (MIC) evaluated on Muller-Hinton agar as described by the Clinical and Laboratory Standards Institute (CLSI). The tetracycline and vancomycin strains were screened by polymerase chain reaction (PCR) for the presence of five common vancomycin and tetracycline resistance determinants, respectively, van A, tet K, tet M, tet L and tet O.
Results: Using the DAD method, 12% of the *Staphylococcus aureus* isolates were resistant to vancomycin and 61/33% to tetracycline. Furthermore, the tet(K) gene was found in 71 isolates, tet(L) in 5 isolates, tet(M) in 30 isolates and tet (O) was detected in one isolate and van(A) was not observed in isolates, by PCR technique.
Conclusion: This study indicates that resistance to tetracyclines is mainly through efflux pumps mediated by tet(K) in *S. aureus* in Sanandaj city. The results of this study can provide guidance for physicians toward more appropriate treatments of *Staphylococcus aureus* infections in Iran, thereby preventing the emergence of further antibiotic resistance among *Staphylococcus aureus*.

Keywords: *Staphylococcus aureus*, Antibiotic Resistance, Tetracycline, Vancomycin

EVALUATION OF RESISTANCE PATTERN OF PNEUMOCOCCAL PNEUMONIA TO CEFTRIAXONE, AZITHROMYCIN AND CO-AMOXICLAV IN CLINIC AND LABORATORY: A TRIAL STUDY

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Background: *Streptococcus pneumoniae* is the most common cause of bacterial infections in the respiratory system. In recent years in addition to the high incidence of pneumococcal resistance to penicillin, resistance to other antibiotics has also emerged. This study was conducted to evaluate the in vivo and in vitro resistance to ceftriaxone, Co-amoxiclav and azithromycin in pneumococcal pneumonia.
Methods: In this clinical single-blind trial study, patients (98 persons) referred to infectious diseases ward of Vali-e-Asr Hospital and to BUMS university clinic diagnosed with pneumonia (during October 2012 - April 2014) were included. Excluded criteria were toxicity; sever illness and antibiotic therapy during the past 48h. The patients were put accidentally on one of the 3 therapeutic regimes including ceftriaxone, Co-amoxiclav and azithromycin and after 48-72 hours when the infection was confirmed by paraclinical findings, the patients with pneumococcal pneumonia remained in the study. In the next step, the in vivo and in vitro resistances to the above-mentioned antibiotics were compared. The data were analyzed by SPSS v.15 software using Chi-squared, Fisher Exact Test and kappa coefficient in a statistical significant level of $p<0.05$.
Results: The results showed the most in vitro drug resistance was to Co-amoxiclav (41.5%) and the least resistance was to ceftriaxone (20.8%). ($P>0.05$). In vivo circumstances the most resistance was to azithromycin (47.4%) and the least one was to ceftriaxone (6.7%) ($p<0.05$). The agreement coefficient between the laboratory antibiogram test and the clinical responses to therapeutic regimes of azithromycin, Co-amoxiclav and ceftriaxone was 0.25 ($p=0.26$), 0.46 ($p=0.02$) and 0.44 ($p=0.04$) respectively.
Conclusion: According to the high susceptibility of *Streptococcus pneumoniae* to ceftriaxone and not having objective complications during the study, this antibiotic is suggested in treatment of pneumococcal pneumonia. Co-amoxiclav is considered as the second treatment option.

Keywords: Drug Resistance, *Streptococcus pneumonia*, Azithromycin, Coamoxiclav, Ceftriaxone

RESISTANCE TO ERYTHROMYCIN AND CLINDAMYCIN OF STAPHYLOCOCCUS AUREUS ISOLATES FROM SANANDAJIAN PATIENTS

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Background: *Staphylococcus aureus* successfully colonizes in humans, contaminates the hospital environment, and has the genetic versatility to acquire resistance to multiple antimicrobial agents. This study was done to determine the prevalence and genetic basis of erythromycin and clindamycin resistance in *Staphylococcus aureus* isolates from Sanandajian patients.
Methods: One hundred and fifty clinical isolates of *S. aureus* were collected from Sanandaj Hospital. Susceptibility to antibiotics (erythromycin and clindamycin) was determined by disk agar diffusion method with minimum inhibitory concentration (MIC) evaluated on Muller-Hinton agar as described by the Clinical and Laboratory Standards Institute (CLSI). The strains *Staphylococcus aureus* were screened by polymerase chain reaction (PCR) for the presence of five common erythromycin and clindamycin genes resistance determinants, which were respectively ermA, ermB, ermC, mphC, msrA.
Results: Using DAD method, 54.6% of the *Staphylococcus aureus* isolates were resistant to erythromycin and 58.6% to clindamycin. The ermA gene was found in 79 isolates, ermB in 36 isolates, ermC in 62 isolates, mphC and msrA were detected in 16 and 29 isolates, respectively, by PCR technique.
Conclusion: This study indicates that resistance to erythromycin is mainly by efflux pumps mediated by ermA and ermC genes in *S. aureus* in Sanandaj city. The results of this study can provide guidance for physicians toward a more appropriate treatment of *Staphylococcus aureus* infections in Iran, thereby preventing the emergence of further antibiotic resistance among *Staphylococcus aureus.*

Keywords: *Staphylococcus Aureus*, Antibiotic Resistance, Erythromycin, Clindamycin

EVALUATION OF CURING AGENTS IN PSEUDOMONAS AERUGINOSA ISOLATES FROM BURNED PATIENTS IN ISFAHAN, Iran

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Background: The present study was conducted to evaluate the antibiotic resistant occurrence of plasmid and to compare the curing agents in *Pseudomonas aeruginosa* isolated from wound of burned patients.
Methods: 220 clinical samples were collected from hospitalized burned patients in Isfahan Burn Hospital within three months (Mar 2012 to Jun 2012). Identification of *Pseudomonas aeruginosa* was performed using standard biochemical tests and then it was confirmed with API 20NE Systems. Antibiotic resistant was determined by standard Agar disk diffusion against three groups of antibiotics viz., meropenem, ceftriaxon, ciprofloxacin. Then plasmid profile was detected by plasmid spin column extraction method, respectively. Furthermore, Plasmid curing was done on this strain using ethidium bromide, rifampin and the elevated temperature.
Results: The results obtained from this study indicated that 60 *Pseudomonas aeruginosa* isolates were resistant to three antibiotics tested. The results showed that the frequency of plasmid curing induced by ethidium bromide was 60%, while high temperature (44°C) and rifampicin couldn not cure the isolated strain. The isolates lost their plasmid after treating with ethidium bromide at 1000 and 2000µg/ml. In the study antibiotics ciprofloxacin, ceftriaxone and meropenem were as resistance markers for plasmid curing. Our finding indicated that 83% and 66% of the cured strains were sensitive to ciprofloxacin and ceftriaxone. However, 33% of the isolates were sensitive to meropenem.
Conclusion: ciprofloxacin, ceftriaxon and meropenem resistant characters probably are plasmid mediated and therefore these genes could transmit among bacteria easily. Some of the resistant *Pseudomonas aeruginosa* isolates were found to lose their resistance to cure agents following treatment with different concentrations of ethidium bromide, rifampin and high temperature in burned patients of Isfahan burned hospital.

Keywords: *Pseudomonas aeruginosa*, Antibiotic Resistance Plasmids, PlasmId Curing

THE SURVEY OF ANTIBIOTIC SUSCEPTIBILITY OF ESCHERICHIA COLI STRAINS ISOLATED FROM CHILDREN WITH URINARY TRACT INFECTIONS BY E-TEST METHOD

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Background: Urinary Tract Infection is one of the most common infections during childhood and *E-coli* is the most predominant pathogen found in UTI. Exact diagnosis and early effective treatment with antimicrobial agents need sensitive and specific methods for detection of antibiotic sensitivity to assist proper treatment and reduce serious renal complications. Therefore, E-test method had been used for detection of Minimal Inhibitory Concentration and our study compares the Disk Diffusion Agar method with E-test in detection of antibiotic susceptibility.
Methods: Our study was conducted in Besat Teaching Hospital on 100 pediatric patients ranged from15 days to 13 years old with positive urine culture for E-coli. Antibiogram detection was performed by Disk Diffusion Agar test with different kits as Padtan-Teb maid in Iran and Mast maid in U.K for Co-trimoxazol, Amikacin, Ceftriaxone, Nalidixic Acid, Cefixime and Nitrofurantoin.MIC was determined using E-test for Co-trimoxazol, Amikacin, Ceftriaxone and Nalidixic Acid.
Results: Co-trimoxazol obtained the lowest and Amikacin had the highest sensitivity in tree methods, which were used in our study. The highest and the lowest overall agreement were found between E-test and Mast Disk Diffusion agar test for Amikacin and Co-trimoxazol, respectively. These results also were compared between E-test and Padtan-Teb Disk Diffusion agar test, which were discovered as the highest, and the lowest overall agreement for Nalidixic Acid and Amikacin respectively.
Conclusion: The more correlation was found between E-test and Mast Disk Diffusion Agar test compared with Padtan-Teb Disk Diffusion Agar test and finally E-test is the most sensitive and specific method in determination of antibiotic susceptibility for E-coli in our study.

Keywords: Antibiotic Susceptibility, Disk Diffusion Agar Test, E-Coli, Urinary Tract Infection, E-Test

MUTATION PATTERN EVALUATION OF HUMAN CYTOMEGALOVIRUS (HCMV) FOR RESISTANCE TO GANCICLOVIR IN Iran IMMUNO-SUPPRESSED PATIENTS DURING 2012-2013

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Background: Human cytomegalovirus (HCMV) remains as an important pathogen for immuno-compromised individuals. Ganciclovir is one of the best drugs used for treatment of this disease, whereas prolonged therapy can cause HCMV resistance to ganciclovir. The main reasons for resistance to ganciclovir drug are mutations in ul97 gene of HCMV.The goal of the study was to evaluate the mutation pattern of HCMV to ganciclovir in immuno-suppressed patients before consuming antiviral medications especially ganciclovir.
Methods: 128 positive clinical HCMV isolated samples for immuno-suppressed patients were selected. 939 bp and 679 bp of ul97 gene were amplified with specific nested PCR primers. The primers were targeted at the highly conserved regions of ul97 gene between the codon numbers of 405 – 607. These codons were correlated to ganciclovir resistance. PCR products of all samples were sequenced. CLC sequence viewer software was employed for analysis of all specimens.
Results: The regions associated to ganciclovir resistance in HCMV existed in the codon numbers of 405, 460, 466, 520, 591, 592, 594, 595, 596, 598, 599, 600, 601, 603, and 607. Evaluation of these codons did not show any mutation. The results of the present study indicated that in the early stage of disease, drug resistance to ganciclovir does not occur. Therefore, ganciclovir consumption can be effective in the early stages. But in contaminated patients having resistant strain of HCMV, protocols of disease should be changed.
Conclusion: The Nested PCR and Sequencing assay are rapid, simple methods for monitoring of ganciclovir resistant Human cytomegalovirus.

Keywords: Human Cytomegalovirus, HCMV, Ganciclovir Resistance.

PREVALENCE OF FLUOROQUINOLONE-RESISTANCE AMONG EXTENDED-SPECTRUM B LACTAMASE PRODUCING KLEBSIELLA ISOLATES FROM CLINICAL SPECIMENS.

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Background: *Klebsiella pneumonia* is one of the most common causes of nosocomial infections causinghigh rate of mortality. However, most isolates of *Klebsiella pneumoniae* are multidrug resistant and increasing the emergence of ESBL-mediated resistance among hospital isolates. Also because ESBL-producing *Klebsiella pneumoniae* are frequently resistant to fluoroquinolone, treatment options for treatment of infections caused by these bacteria are limited. The aim of this study was to determine the prevalence of fluoroquinolone-resistance among Extended-Spectrum β-Lactamase producing *Klebsiella* isolates from clinical specimens referred to Shahid Beheshti hospital in Kashan.
Methods: A cross-sectional study was conducted between April 1, 2013, and April 17, 2014. 121 non-repetitive clinical isolates suspected of *Klebsiella* from patients referred to Shahid Beheshti hospital in Kashan, were enrolled in the study. Antimicrobial resistance profile and detection of quinolone resistance were determined using disk diffusion method according to the Clinical and Laboratory Standards Institute. Double disk synergy test was performed to confirm the production of Extended-Spectrum β-Lactamases (ESBLs). ESBLs-producing *Klebsiella* showed to be resistant to fluoroquinolone was investigated using the disk diffusion method and the double-disk synergism test.
Results: Of the 121 *Klebsiella pneumoniae* isolates, 83(68.6%) were nosocomial. *Klebsiella pneumoniae* were isolated from urine 78 (64.5%), wound 6 (4.9%), blood 4 (3.3%), respiratory 30 (24.7%), CSF 1(0.82%), catheter 2(1.65%). The mean age of the patients was 50 years (male 47, female52). Among the 121 clinical isolates of *Klebsiella pneumoniae*, 58(48%) were identified as ESBL producers. Of these ESBL positive *Klebsiella pneumoniae* isolates, 50(86.20%) were fluoroquinolone resistant of which 50 (100%) demonstrated multidrug-resistant pattern. The most prevalence of resistance was seen against ceftazidime, cephalothin and ampicillin among ESBL positive, fluoroquinolone resistant *Klebsiella pneumoniae* isolates.
Conclusion: The findings of this study demonstrated a high prevalence of fluoroquinolone resistance in patients with ESBL *Klebsiella* infections. Our results suggest that strategies must be used to limit the overuse of fluoroquinolone in such patients and preserve the usefulness of fluoroquinolones in treatment of such infections.

Keywords: *Klebsiella pneumoniae*, ESBL, Multi Drug Resistance, Fluoroquinolone Resistance

AN INVESTIGATION OF FREQUENCY AND ANTIMICROBIAL SUSCEPTIBILITY OF PULMONARY PATHOGENS IN CYSTIC FIBROSIS (CF) PATIENTS IN ALZAHRA HOSPITAL IN ESFEHAN.

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Background: This study was performed to investigate the frequency and antimicrobial susceptibility of pulmonary pathogens in cystic fibrosis (CF) patients.
Methods: 129 pediatric patients with CF were enrolled in this cross-sectional study.Microbiological cultures were performed based on sputum or pharyngeal swabs. Antibiotic susceptibilities of the isolated bacteria were determined by the disk diffusion method.
Results: The main infecting pathogens were *Pseudomonas aeruginosa* (48.8%), Klebsiellapneumoniae (13.7%) and Staphyloccus areus (10.3%), respectively. The most active antibioticsincluded rifampin (93.7% susceptibility), vancomycin (88%) and imipenem (86.5%). The emergence of resistance against aminoglycosides was observed.
Conclusion: Regarding in vitro susceptibility results, cyclic treatment of long-term oralazithromycin and inhaled tobramycin could prophylactically be applied, and duringexacerbations, imipenem or ceftazidime in combination with an aminoglycoside such asamikacin could be considered as choices for treatment.

Keywords: Antimicrobial Susceptibility, Cystic Fibrosis

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THE PATTERN OF RESISTANCE IN ENTEROCOCCI ISOLATED FROM NOSOCOMIAL INFECTIONS IN HOSPITALS GONBAD AND GORGAN CITIES

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Background: *Enterococci* are normal flora of human body parts. They are known as the second leading cause of nosocomial infections. The purpose of this study was to determine the drug resistance of *Enterococcus* species through biochemical methods.
Methods: 128 samples were *Enterococcus* suspected. They were isolated from April to June 2012 in hospitals of Gorgan and Gonbad. Samples were cultured on the blood agar chrome-agar and EMB agar. Suspensions of bacteria were grown in Mueller Hinton agar mediumand. The antibiotic susceptibility was determined by disk inhibition zone diameter.
Results: From 128 cases, 109 cases (85/15%) were *Enterococcus faecalis* and 19 (14/85%) were *Enterococcus faecium*. Isolates of *Enterococcus faecalis* showed resistance to amoxicillin, 6 (5/5%), ampicillin 7 (6/4%), gentamicin 4 (3/66%), ciprofloxacin 3 (2/75%), chloramphenicol 4 (3/66%), cephalexin 3 (2/75%) and vancomycin 8 (7/34%), respectively. Of 19 samples of *Enterococcus faecium*, resistance to amoxicillin in 2 (5/10%), ampicillin 3 (78/15%), gentamicin 1 (26/5%), ciprofloxacin 2 (5/10%), chloramphenicol2 (5/10%), cephalexin 1 (26/5%) and vancomycin 3 (78/15%) was observed.
Conclusion: Resistance reported in this study has many similarities with other researches. Faecalis higher percentage than Faecium of the main results obtained. There is more than one resistance among *Enterococci* showed resistance to more than one antibiotic.

Keywords: *Enterococci*, Antibiotic Resistance, Antibiotic Susceptibility

CHARACTERIZATION OF SUBTYPES OF CTX-M EXTENDED-SPECTRUM B –LACTAMASE AMONG KLEBSIELLA SPP

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Background: The CTX-M family consists of more than 20 β-lactamases which were on the basis of sequences divided into 4 subtypes including CTX-M-1, CTX-M-2, CTX-M-8 and CTX-M-9. The aim of current study was to detect the subtypes of CTX-M ESBLs among ESBL positive *Klebsiella* isolates from patients in Kashan.
Methods: A total of 100 clinical isolates of *Klebsiella* were collected during December 2012 to November 2013. The *Klebsiella* isolates which showed resistance or reduced susceptibility to cefotaxime, ceftazidime and/or aztreonam by disk diffusion method were selected. These isolates were identified as ESBL-producing isolates by double disk synergy tests using clavulanic acid, cefotaxime, ceftazidime and aztreonam. The blaCTX-M subtypes determinants were identified by PCR method followed by DNA sequencing.
Results: Of the 100 *Klebsiella* isolates, 41% (n=41) demonstrated resistance or reduced susceptibility to ceftazidime and/or aztreonam and 35% (n=35) were ESBL-producers. Twenty-eight (8o %) of ESBL-producing isolates carried blaCTX-M type genes. Based on PCR assays and sequencing of blaCTX-M genes, CTX-M-1, CTX-M-2 and CTX-M-9 were identified in 21(60%), 15(42%) and 9(34%) of these isolates respectively (GenBank accession numbers KJ803828-KJ803829). CTX-M-8 was not found in isolates and both CTX-M-1 and CTX-M-2 were identified in 8 (22%) of ESBL-producing isolates.
Conclusion: Our study showed that the frequency of blaCTX-M genes among *Klebsiella* isolates in our region is at alarming rate. Also we found a high prevalence of blaCTX-M-1 β-lactamase in *Klebsiella* isolates in kashan indicating blaCTX-M-1 β-lactamase as a significant group of ESBLs in this region.

Keywords: Blactx-M, *Klebsiella* Spp, Extended-Spectrum ß–Lactamase (Esbls), Clinical Specimens

THE FREQUNCY OF EXTENDED-SPECTRUM BETA-LACTAMASE-PRODUCING ESCHERICHIA COLI AT AN EDUCATIONAL HOSPITAL IN SHAHREKORD, Iran.

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Background: The current study showed the prevalence of extended-spectrum beta-lactamase–producing *Escherichia coli* uropathogens in Shahrekord, Iran.
Methods: A total of 200 urinary tract infections (UTIs) of *E. coli* were collected from an educational hospital in shahrekord, Iran. Uropathogens were identified through culture, microscopy and biochemical tests. The agar disc diffusion assay was used to determine the antimicrobial susceptibilities of uropathogens. The ESBL positive strains were further subjected to phenotypic confirmatory tests using sensitivity discs, which contained third-generation cephalosporins both with and without clavulanic acid. The discs (ceftazidime (30 μg), ceftazidime + clavulanic acid (30 μg + 10 μg). extended-spectrum beta-lactamase resistance gene VEB were examined in ESBL positive strains using by PCR.
Results: Combined double disc synergy test was applied to detect ESBL in 90 *E. coli* isolates that are resistant to ceftazidime using ceftazidime alone or with clavulanic acid. Among the 90 ESBL-producing *E. coli* strains, 10(11.11%) were identified to produce VEB enzyme.
Conclusion: UTIs are one of the most frequently encountered conditions in clinical medical practice requiring antimicrobial therapeutic intervention. To date, *E. coli* has been the most common isolated pathogen causing UTIs. ESBL-producing *E. coli* is now distributed in the world and its prevalence is increasing in community-acquired infections. ESBL-producing *E. coli* isolates have also become a serious problem in clinical setting.

Keywords: Esbls, E. *coli*, VEB

PREVALENCE OF OXA-1 TYPE ESBLS AMONG CLINICAL ISOLATES OF ENTEROBACTER CLOACAE COLLECTED FROM QAZVIN HOSPITALS, Iran

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Background: The aim of this study was to determine the frequency of OXA-types among ESBL-producing *E. cloacae* isolates collected from Qazvin hospitals.
Methods: Totally, 61 ESBL-producing *E. cloacae* isolates were collected from different clinical samples. Species` identification was performed using standard laboratory methods. ESBL production was confirmed by phenotypic combined disk method as recommended by the Clinical and Laboratory Standards Institute (CLSI). PCR and sequencing were performed for detection of blaOXA-1, blaOXA-2, blaOXA-9 and blaOXA-10 genes using specific primers.
Results: In total, 34(55.7%) of ESBL-producing isolates were positive for the presence of blaOXA-1 genes. blaOXA-2, blaOXA-10 and blaOXA-9 genes were not detected in this study. All blaOXA-1 -producing isolates showed multidrug resistant pattern. blaOXA-1-producing isolates were mostly recovered from wound (32.4%), followed by trachea (23.5%) samples. These isolates were mostly collected from patients admitted in ICU (44.1%) and Internal medicine (7.6%) wards.
Conclusion: The results of this study showed a considerable prevalence of resistance due to the blaOXA-1 genes among the clinical isolates of *E. cloacae* in Qazvin hospitals. The emergence and spreading of these resistant determinates in our clinical setting increase serious problem for infection control management and antibiotic therapy, which highlight the need for adopting appropriate infection control policy.

Keywords: *Enterobacter cloacae*, Blaoxa-1, Esbls

PLASMID-MEDIATED QUINOLONE RESISTANCE DETERMINANTS AMONG ENTEROBACTER CLOACAE ISOLATED FROM HOSPITALS OF QAZVIN, Iran

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Background: Plasmid-mediated quinolone resistance is the new mechanism of quinolone resistance, which has been reported in several parts of the world. Three major groups of qnr determinants, qnrA, qnrB, and qnrS, are increasingly being identified in clinical isolates of various enterobacterial species. The main aim of this study was to determine the frequency of qnr genes among clinical isolates of *E. cloacae* collected from Qazvin hospitals.
Methods: A total of 96 nalidixic acid non-susceptible *E. cloacae* isolates were obtained from different clinical samples. Antimicrobial susceptibility was determined by standard Kirby-Bauer disk diffusion method. PCR and sequencing were employed to detect qnrA, qnrB, and qnrS genes using the specific primers.
Results: In total, qnr-encoding genes were present in 56 (58.3%) nalidixic acid non-susceptible isolates of which qnrB1 (36-45%) was the most common gene followed by qnrS1 (22-27.5%), and qnrB4 (15-18.8%) either alone or in combination, respectively. qnr-producing isolates were mostly recovered from wound (20-35.7%), followed by trachea (11-19.6%) samples. These isolates were mostly collected from patients admitted in ICU (24-42.9%) and Internal medicine (15-26.9%) wards.
Conclusion: This study for the first time showed the high emergence of qnrB1,qnrS1 and qnrB4 genes among *E. cloacae* isolates in Iran. This study emphasize the need for adopting appropriate infection control policy and rational antibiotic therapy to reduce further spread of these resistant bacteria in our hospitals.

Keywords: Enterobacter *cloacae*, Qnr, Quinolone

THE PREVALENCE AND DISTRIBUTION OF AADA1, DHFRA1-AADA1 AND DFRA1 GENE CASSETTES OF CLASS 2 INTEGRON AMONG CLINICAL ISOLATES OF STAPHYLOCOCCUS AUREUS FROM HOSPITALS IN TEHRAN: THE FIRST REPORT

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Background: The aims of this study were to determine antimicrobial susceptibility, to detect class 2 integrons in isolates of *Staphylococcus aureus* and to analyze genes cassettes content of class 2 integrons in selected multi-drug resistance (MDR) strains.
Methods: 139 *S.aureus* isolates were collected from hospitals in Tehran. Antimicrobial susceptibility testing (Streptomycin, Tetracycline, Sulfamethoxazole-Trimethoprim, Gentamicin, Vancomycin, Oxacillin, Levofloxacin, Erythromycin, Spectinomycin, Amoxicillin, Ciprofloxacin) was done for MDR isolates by disk diffusion method (Kirby-bauer). *E. coli* ATCC 25922 was used as the quality control organism in antibiogram test. The DNA was extracted with phenol-chloroform and class 2 integrase (intI2) genes were detected by PCR. intI2-positive isolates were further analysed for the presence of resistance gene cassettes using specific primers 5'cs/3'cs.
Results: Among 139 *S.aureus* isolates, 109 (78.4%) strains were considered as MDR. PCR assays (dintI2 gene and internal variable regions (IVRs) of class 2 integron in 49/139 (35.2%) and 32/49 (65.3%) of *S.aureus* isolates respectively. Analysis of the sequence data reveald 3 gene cassette arras deposited in Genbank databases including the dhfr1-aadA1 (1583bp), aadA1 (789bp) and ahfrA11 (474bp) with 3 IVRs distribution patterns.
Conclusion: This is the first report of class 2 integrons in clinical S.aureus. These results indicated with the spread of MDR strains, class 2 integron carrying gene cassettes are widely disseminated among *S.aureus* strains in hospital. These structures are playing a major role in the acquisition of multidrug resistance in these strains.

Keywords: Integron, *Staphylococcus aureus*, MDR, Gene Cassette

KLEBSIELLA PNEUMONIAE, B-LACTAMASES, 16S RRNA METHYLASE, ARMA, BLACTX-M-15

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Background: The emergence of antibacterial resistance and ESBL-producing *Escherichia coli* recovered from urinary tract infections (UTI) areknown as important health problems in different regions, therefore therapeutic options for these infections are limited.The aim of this study was the detection of blaTEM, blaSHV and blaCTX-M genesamong *Escherichia coli* strains isolated from Children Medical Center hospital, Tehran, Iran.
Methods: This study was conducted on 100 *Escherichia coli* isolates from urine specimens of patients with UTI who referred to Children Medical Center, Tehran, Iran from November 2012 and July 2013. Antibiotic susceptibility test was performed by Kirby-Bauer disc diffusion method according to CLSI guidelines. The blaCTX-M, blaTEMandblaSHVgeneswere detected by PCR and sequencing methods.
Results: The resistance rate of isolates toGentamicin,Imipenem, Nitrofurantoin,Cefotaxime, Ceftazidime, Amikacin, Cefepime, Piperacillin/Tazobactam, Cotrimoxazole,Cefixime andCephalothin, were 25 (25%),0 (0%), 7 (7%), 51(51%), 24(24%), 2 (2%),6 (6%),2 (2%),79 (79%),36 (36%) and 43 (43%)respectively.In this study, imipenemwas more active than other antibiotics.The existence of blaTEM-1 and blaCTX-M-15was detected in 69 (69%) and 74 (74%) of isolates respectively, while blaSHVgene wasnot detected.
Conclusion: The prevalence of ESBL-producing *E. coli* detected in this study is of great concern and highlights the need of infection control measures including antibacterial management and prompt identification of ESBL-producing isolates.

Keywords: *Escherichia coli,*UTI, Children, Esbls

DETECTION OF THE ANTIBIOTIC RESISTANCE PATTERN IN STAPHYLOCOCCUS AUREUS ISOLATED FROM URINARY TRACT INFECTIONS IN MARAND CITY

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Background: *Staphylococcus aureus* is considered as a common pathogenic factor in infections. The increase in rates of infections caused by this bacterium in developing countries has led to many problems. The purpose of this study was to determine the prevalence of antibiotic resistants of *Staphylococcus aureus* strains isolated from urinary tract infections to some commonly used antibiotics.
Methods: In this cross-sectional study, 45 *S. aureus* isolated from urinary tract infections of the patients. These strains were selected using laboratory standard methods and culture-specific. The antibiotic susceptibility testing was performed using Kirby-Bauer disk diffusion method.
Results: Based on the phenotypic investigation on antibiotic resistance of *S. aureus* strains, the highest rates were resistance with penicillin (95.6%), tetracycline (88.89%), and sensitive to Vancomycin (100%) and Clindamycin (91.1%).
Conclusion: This study showed increased resistance to different antibiotics in *Staphylococcus aureus* that is a serious warning to the treatment of infections caused by this bacterium in the region. Therefore, in order to prevent increased resistance to other antibiotics, it is essential to withhold prescriptions and unnecessarry use of available antibiotics.

Keywords: *Staphylococcus aureus*, Urinary Tract Infection, Antibiotic Resistance

INVESTIGATING THE EFFECT OF COTRIMAXAZOL AS PROPHYLAXIS FOR PREVENTING URINARY TRACT INFECTION CAUSED BY KLEBSIELLA AFTER THE CESAREAN SECTION IN HOSPITALS OF SOUTH OF Iran

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Background: Since one of the infections that might occur after the cesarean section is the urinary tract infections, this study aims at investigating the general prevalence of this kind of infection caused by Cotrimaxazol-resistant *Klebsiella*, as well as determining the effective genes on making resistance by PCR. It is also going to suggest appropriate treatment strategies in order to reduce treatment costs and drug reactions in patients.
Methods: We first took notes from the results of the U/A tests done on cesarean sectioned mothers in the hospitals and, as this study aims at preventing drug therapy without doing suitable tests, a urine analysis test was prescribed by the surgeon to be done 16 days after the surgery in order to find out whether the U/A tests alone can help doctors prevent the infection by doing post-operation prophylaxis based on their experiences. Hence, the patients' sterile median urine samples taken 16 days after the surgery were collected in the laboratory. The samples were cultured within 20 minutes and the cultures were put into the incubator at 37 °C for 24 hours. When the cultures became positive, the colonies type was detected using a diagnostic test and the antibiotic sensitivity of the colonies was investigated through the Antibiogram test.
Results: Only 2 out of 143 people (1.4%) who referred to the laboratories were infected by urinary tract infection caused by *Klebsiella pneumonia*, one of which (0.7%) was found to be resistant to Cotrimaxazol while the other one was sensitive to this drug. The most amount of antibiotic-resistance of those two samples (100%) was related to Cefixime, Cefazolin, Cefalexin, Ceftriaxone, and Ciprofloxacin, while the least amount of resistance was related to Gentamicin and Nitrofurantion (0%). The molecular analysis of the genes causing resistance showed that the Cotrimaxazol-resistant isolate had both SUL1 and SUL2 genes with 100% frequency, while the sensitive isolate had neither SUL1 nor SUL2 genes.
Conclusion: To prevent the creation of drug-resistant strains, doctors must prescribe antibiotics and post-surgery prophylaxis after doing accurate tests. Besides, the insurance companies should support patients for paying the test expense because wrong drug therapy imposes extra costs on patients and the government as well.

Keywords: Antibiotic-Resistance, Cotrimaxazol, Cesarean Section, Integron, SUL1 And SUL2.

PREVALENCE OF SULFONAMIDE RESISTANCE GENES IN SALMONELLA SPP. ISOLATED FROM MILAD HOSPITAL OF TEHRAN

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Background: Extensive use of antimicrobial agents such as Cotrimoxazole has been associated with rising of antimicrobial resistance. In the current study, we focused on assessing the prevalence of *Salmonella* spp. resistance to Cotrimoxazole and the frequency of its genes.
Methods: Forty- nine *Salmonella* spp. isolates were identified from Mar.2011 to Apr.2013 in Milad hospital of Tehran. The antibiotic susceptibility test for screening the resistant isolates was done by Kirby-Bauer method. The sul1, sul2, sul3, dfrA1, dfrA5, and Int1, genes were detected by Multiplex-PCR amplification.
Results: Among 49 *Salmonella* isolates, the frequency of sul1 was 62.5(10 isolates) and the number of other genes were; dfrA1: 25%(4 isolated), dfrA5: 18.75(3 isolated), sul1: 81.25(13 isolated), sul2: 56.25(9 isolated), and sul3: 0%(none).7 isolates had sul1 and sul2 simultaneously (43.75%), and 3 isolates (18.75%) had int1 and dfrA1.
Conclusion: Our study illustrated high frequency of Cotrimoxazole resistance genes in Milad hospital of Tehran. Sul genes have major roles in Cotrimoxazole resistance of *Salmonella* isolates.

Keywords: Cotrimoxazole, Dfr, Int, Sul, Antibiotic Resistance, *Salmonella* Spp., Tehran

REPETITIVE ELEMENT PCR FINGERPRINTING (REP-PCR) IN KLEBSIELLA PNEUMONIAE PRODUCING CTX-M IN SANANDAJ

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Background: 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. The aim of this study was to determine the prevalence of CTX-M and associated risk factors for community-acquired *Klebsiella pneumoniae* in Sanandaj, Iran.
Methods: In this study, 100 *Klebsiella pneumonia* strains community-acquired were used. The pattern of antimicrobial resistance was determined by double disk diffusion method. The ESBL production was determined by combination disk method using disks containing Cefepime, Cefpodoxime, ceftazidime and cefotaxim alone and in combination with Clavulanic acid. CTX-M type of ESBL producing genes were detected by PCR. A possible clonal relationship among the strains was determined by repetitive extragenic palindromic sequence 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. Base on rep-PCR, 31 genotypes among 37 CTX-M-positive samples were detected. According to statistical analysis, the followings were identified as independent risk factors: age (P value: 0.006, 95%CI: 2.613-15.084), pregnant (P value: 0.036, OR: 5.903, 95%CI: 1.125-30.975), previous hospitalization in the past 3 months (P value< 0.001, OR: 11.96, 95%CI: 4.541-31.491), time of hospitalization (P value< 0.001), antibiotic treatment in the past 3 months (P value: 0.016, OR: 2.806, 95%CI: 1.208-6.518), having relatives in hospital staff (P value: 0.001, OR: 12.904, 95%CI: 2.671-62.336), Diabetes Distance under 2 km from the hospital.
Conclusion: Noticing the increasing rate of the ESBLs producing strains, using the appropriate treatment protocol based on the PCR pattern of the strains is highly recommended. Hospitals and hospital staff should have more hygiene, proper disposal of hospital waste and the use of antibiotics only if prescribed by a doctor can help prevent the spread of ESBL.

Keywords: CTX-M Genes, *Klebsiella pneumonia*, Extended-Spectrum B- Lactamases

FREQUENCY OF PER,VEB,SHV, TEM AND CTX-M GENES IN RESISTANT STRAINS OF PSEUDOMONAS AERUGINOSA PRODUCING EXTENDED SPECTRUM ß-LACTAMASES ISOLATED FROM CLINICAL SPECIMENS IN EDUCATIONAL HOSPITALS OF ZAHEDAN

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Background: *Pseudomonas aeruginosa* is the most common pathogen causing nosocomial infections. Resistance of P. aeruginosa strains to the broad-spectrum cephalosporins may be mediated by the extended-spectrum b-lactamases (ESBLs). This study aimed to investigate the prevalence of ESBLs and antimicrobial susceptibilities of P. aeruginosa isolated from patients in Zahedan, Iran.
Methods: A total of 116 isolates of P. aeruginosa were collected during a year from teaching hospital in Zahedan, Iran. Susceptibility to nine antimicrobial agents was performed by disk diffusion method. ESBL producing strainswere detected by Combination disk test (CDT). In this study ESBL positive and other isolates showing MICs ≥ 4 µg/ml for ceftazidime, Cefotaxime, Ceftriaxone and Aztreonam were screened for the presence of the genes encoding bla(TEM), bla(SHV), bla(PER-1) and bla(VEB-1), genes by polymerase chain reaction.
Results: Ciprofloxacin and piperacillin were the most effective anti-pseudomonal agents. The results revealed that 19 (16.37%) of the isolates were multidrug resistant and 8 (6.89%) of the isolates were ESBL positive. Of a total of 116 isolates, 30 (25.86%) were at least resist to one of the antibiotics ceftazidime, ceftriaxone, cefotaxime and Aztreonam and among this 30(100%), 4(13.3%), 2(6.6%) and 2(6.6%), amplified blaTEM, blaVEB-1, blaPER-1 and blaSHV respectively. 22 out of 30 isolates of TEM-positive isolates showed ESBL-negative phenotype. Sequencing of the ESBL genes confirmed the genuinety of PCR products.
Conclusion: From results of the present investigation it can be concluded that blaTEM is the most frequent isolated ESBL gene among *P.aeruginosa* strains isolated from patients.

Keywords: *Pseudomonas aeruginosa*, Extended-Spectrum Beta-Lactamase

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DETERMINATION THE FREQUENCY OF ADERS GENES AMONG CLINICAL ISOLATES OF CARBAPENEM RESISTANCE A. BAUMANNII STRAINS

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Background: Carbapenems are considered as the key treatment in many cases of nosocomial infections of *Acinetobacter baumannii*. AdeABC efflux pump is the first characterized resistance–nodulation–cell division (RND) systems in resistant *A. baumannii* and tightly is regulated by the two-component system AdeS /R. So, the aim of this study was to determine the frequency of adeS and adeR genes among carbapenem resistant *A. baumannii* clinical isolates.
Methods: 60 clinical carbapenem resistant *A. baumannii* isolates were obtained from Taleghani and Motahari hospitals in Tehran during 2013. Antimicrobial susceptibility testing was done by Kirby-Bauer method and MIC of imipenem was determined by the microdilution method, according to CLSI 2012. Simultaneously, *A. baumannii* ATCC27853 was evaluated as the reference strain. DNA extraction was done by boiling method and the frequency of the above genes was detected by PCR.
Results: 34 out of 60 A. baumanni strains were isolated from burn wounds of Motahari hospital and 19 from trachea(13), blood(6) of ICU as the frequent ward of Taleghani hospital. Based on the antibiotic resistance test, the most resistance rates were detected to Ceftriaxone, Cefotaxime, Ciprofloxacin, Trimethoprim-sulfamethoxaxole, Ceftazidime, Piperacillin, Piperacillin-tazobactam (100%) and all isolates (100%) were sensitive to colistin. Also based on antibiogram and MIC 52(86.6%) of isolates were resistance to Imipenem. The frequency of adeR/ adeS was detected in 36 (60%) and 59 (98.3%), respectively.
Conclusion: The existence of adeS/R genes in more than half of A.baumanni isolates with nodifference in the origin of sample, shows the importance of efflux pumps in carbapenem resistant A. baumanni strains.

Keywords: *Acinetobacter baumannii*, Aders, Imipenem

DRUG SUSCEPTIBILITY PATTERN OF DIFFERENT ANTIMICROBIALS ON METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS, ISOLATED FROM PATIENTS ADMITTED TO KHATAM HOSPITAL

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Background: The aim of this study was to determine antibiotic sensitivity pattern of HA-MRSA and conduct on new antibacterial agents.
Methods: For this study 150 *S.aureus* strains were isolated from patients of Khatam hospital in Tehran, Iran from Januarys 2013 to June 2014. Sterilized nasal swabs were used to collect nasal specimen. Nasal isolates were further recognized as *S.aureus* strains by standard biochemical tests, and disk diffusion method was used to determine their methicillin resistance pattern. Susceptibility to antimicrobial agents was performed on MRSA strains for 7 antibiotics including gentamicin, vancomycin, erythromycin, chloramphenicol, rifampin, linezolid and teicoplanin according to CLSI guidelines. Then, susceptibility of screened isolates exposed tea tree oil and methanol extract of *Satureja khuzestaniea* was assayed by agar well diffusion method.
Results: Our results revealed that the prevalence of MRSA isolates was 36%. Resistance rates of MRSA to various antibiotics were as follows: erythromycin (61%) and gentamicin (50%).Also high susceptibility rates to vancomycin (100%), chloramphenicol (90%), rifampin (94%), linezolid (100%) and teicoplanin (100%) were documented. In this study it was found that methanol extract of *Satureja khuzestaniea* and tea tree oil have anti-bacterial properties but anti-bacterial effects of tea tree oil were more than other and effective as well as vancomycin, teicoplanin and linezolid and the MIC was found to be 12.5%.
Conclusion: Plant extracts, especially methanol extract of *Satureja khuzestaniea* and tea tree oil have antibacterial effects on MRSA strains as well as antibiotics described above, so these compounds could be used in medical treatments.

Keywords: MRSA, Antibiotic Susceptibility, Plant Extract,

HIGH FREQUENCY OF MBL PRODUCING ACINETOBACTER IN KERMANSHAH

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Background: *Acinetobacter* is a gram-negative non-fermentative coccobacilli with increasing prevalence in a variety of hospital-acquired infections.The aim of this study was todetermine the frequency of extended spectrum β-lactamase (ESBL) and metallo-β-lactamase (MBL) in *Acinetobacter* isolated from clinical specimens.
Methods: 100 isolates of *Acinetobacter* were collected from different clinical samples including lood, wound, and liquid specimens of hospitalized patients of Imam Reza and Taleghani hospitals in Kermanshah. All isolates were identified by standard biochemical tests. ESBL and MBL producing strains were identified by phenotypic method of combined-disk ceftazidime/clavulanic acid and imipenem/EDTA.
Results: Among 100 *Acinetobacter* isolates 58 (58%) were resistant to imipenem and 94 (94%) were resistant to ceftazidime. In total, 10% of isolates were ESBL-positive and 72% of them were positive for MBL.
Conclusion: The result shows MBL producing *Acinetobacter* is more prevalent in Kermanshah compared to other cities. However the carbapenems is one the excellent treatments against infections caused by multidrug-resistant *Acinetobacter*, but high prevalence of MBL and ESBL producing *Acinetobacter* dramatically reduces the existing therapeutic options and poses a potential threat to public health.

Keywords: *Acinetobacter*, Disk Diffusion, ESBL, MBL.

ANTIMICROBIAL RESISTANCE AND CLASS 1 INTEGRON IN ESCHERICHIA COLI STRAINS ISOLATED FROM DIFFERENT ANIMAL SOURCES

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Background: The aim of the current study was to evaluate the prevalence of class 1 integrons associated antibiotic resistance among *E. coli* strains isolated from different animal sources.
Methods: *E. coli* strains isolated from different animal sources including human, hen, cow and sheep were included in this study. Bacterial strains were isolated and identified by standard microbiological and biochemical tests. The antimicrobial susceptibility testing was performed according to Kirby Baur assay using 11 antibiotic discs including Amikacin, Co-trimoxazole, Tobramycin, Stereptomycin, Piperacillin, Ampicillin, Cefazolin, Nalidixic acid, Gentamicin, Kanamycin, and Neomycin. Total genomic and plasmid DNAs were extracted using AccuPrep® Genomic DNA Extraction Kit. To detect the class 1 integron, a newly designed int1 specific primer was used for amplification of integrase gene by PCR. The PCR amplicons were visualized after electrophoresis and staining with SYBR green.
Results: Eighty *E. coli* strains were isolated and included in this study. Antibiotic susceptibility testing showed that 59% of the isolates were MDR while 31% harbored the int1gene. Following statistical analysis, chi–square test and p value determination, we found a significant relation between the presence of class 1 integron and resistance to Cefazolin, Sulfamethoxazole, Nalidixic acid, Gentamicin and Kanamycin resistance.
Conclusion: Result from current research indicates high prevalence of antibiotic resistant *E. coli* strains isolated from different animal sources. Our results also confirm the significant role of class 1 integron to create and facilitate the spread of antibiotic resistance among *E. coli* strains.

Keywords: *E. coli*, Class 1 Integron, Antibiotic Resistance.

PREVALENCE OF CTX-3 (CTX-M 3,15,22) FAMILY GENE IN VARIOUS E.COLI AND KLEBSIELLA PNEUMONIAE CLINICAL SPECIMES IN TABRIZ

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Background: Beta-lactam antibiotics are the most frequently prescribed antibiotics. The emergence of resistance to these antibiotics among gram-negative bacilles has limited their efficiency. This study was done to determine the frequency of producing ESBLs, pattern of resistance and presence of CTX-M3 family gene that contain CTX-M 3,15,22 subfamily in *E. coli* and *Klebsiella pneumoniae* isolated from different specimen in Sina Hospital of Tabriz
Methods: This study was done on 71 strains of *E. coli* and 63 strains of K.pneumoniae isolated from Microbiology laboratory of Sina Hospital in Tabriz, Iran. Bacteria were identified by conventional biochemical and phenothipic testes. Susceptibility testing on Muller Hinton was performed with agar disk diffusion method. ESBL producing phinamina in *E. coli* and K.pneumoniae was detected with combine disc method in our study. Finally the presence of CTX-M3 gene was detected by PCR technique and the CTX-M3 family genes were conformed by sequencing.
Results: 41 (57.74%) of *E. coli* and 45 (71.42%) of *K.pneumoniae* isolates were ESBL producing. Among the ESBL- prodiucing *E. coli* 30 (73.17%) and among the ESBL - prodiucing *K.pneumoniae* 26 (57.77%) of CTX-M3 gene was positive. Antibiotic resistance in *E. coli* was: cefpodoxime 66(92%), Aztreonam 56 (78%), ceftazidime 47(66.1%), cefotaxime 49 (69%)), cefepime 20(28%).The resistance pattern in K.pneumoniae was: cefpodoxime 57(90%), Aztreonam 57(90%), ceftazidime 55(87%), cefotaxime 54(85%), cefepime 15(23%)
Conclusion: Our study revealed that there is a high frequency of ESBls producing strains of *E. coli* and K.pneupmia.This has a significant implication for patients` management.It is necessary for further drug resistance surveillance in our hospitals and molecular characteristics ESBls isolates in our country.

Keywords: *Klebsiella pneumoniae*, CTX-3, *E.coli*

DETERMINATION OF CLASS 2 INTEGRON ASSOCIATED ANTIBIOTIC RESISTANCE IN ESCHERICHIA COLI STRAINS

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Background: The aim of the current study was to determine class 2 integron associated antibiotic resistance in *Escherichia coli* strains isolated from four animal sources.
Methods: *E. coli* strains were isolated from different animal sources including human, hen, cow and sheep. Bacterial strains were isolated and identified by standard microbiological and biochemical tests. The antimicrobial susceptibility testing was performed according to Kirby Baur assay using 11 antibiotic discs including Amikacin, Co-trimoxazole, Tobramycin, Stereptomycin, Piperacillin, Ampicillin, Cefazolin, Nalidixic acid, Gentamicin, Kanamycin, and Neomycin. Total genomic and plasmid DNAs were extracted using Accu-Prep® Genomic DNA Extraction Kit. To detect the class 2 integron, a newly designed int2 specific primer was used for amplification of integrase gene by PCR. The PCR amplicons were visualized after electrophoresis and staining with SYBR green.
Results: Eighty *E. coli* strains were isolated and included in this study. Antibiotic susceptibility testing showed that 59% of the isolates were MDR while 10% harbored the int2gene. Following statistical analysis, chi–square test and p value determination, we found significant association between the presence of class 2 integron and resistance to Streptomycin,Sulfamethoxazole and Kanamycin.
Conclusion: Our findings showed that class 2 integrons have significant distribution among *Escherichia coli* strains in different animal sources. Specific care and control on antibiotical resistance including screening of integrons are important strategies to prevent the spread of antibiotic resistance in E.coli.

Keywords: *E. coli*, Class 2 Integron, Antibiotic Resistance.

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THE STUDY OF QNRS ASSOCIATED ANTIBIOTIC RESISTANCE AMONG ESCHERICHIA COLI STRAINS ISOLATED FROM DIFFERENT WATER SOURCES IN ALBORZ PROVINCE

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Background: *Escherichia coli* is a frequent cause of life threatening bloodstream and other common infections such as urinary tract infections, while water can be a great source of bacterial transmission. Antibiotic resistance rates especially against fluoroquinolones in *E. coli* are rapidly rising. Antibiotic-resistant bacteria such as *E. coli* released from humans and animals into water sources may act as donors of antimicrobial resistance genes to other pathogenic *E. coli*. The aim of this study was to investigate the prevalence of qnrS associated antibiotic resistance among *E. coli* strains isolated from different water sources in Alborz province.
Methods: The study included all *E. coli* strains isolated from different surface water sources in Alborz province in 2013. Bacterial strains were isolated, detected and identified by standard microbiological tests. The antimicrobial susceptibility testing was done according to Kirby Baur assay. Total genomic and plasmid DNAs were extracted by boiling method. All Nalidixic acid and ciprofloxacin resistant strains were examined for the presence of qnrS genes by PCR. The PCR amplicons were visualized after electrophoresis and stained with ethidium bromide.
Results: One hundred *E. coli* strains were isolated from water source and examined in this study. Antibiotic susceptibility testing showed that 22.44% and 7.14% of the isolates were resistant to Nalidixic acid and Ciprofloxacin. qnrS gene was detected in 21 fluoroquinolone-resistant isolates. The remained fluoroquinolone-resistant isolates and all fluoroquinolone-susceptible isolates did not contain qnrS gene.
Conclusion: An increasing prevalence of fluoroquinolones resistance was observed among the waterborne *E. coli* strains. These findings reinforce the message that control of the spread of antibiotic resistance requires the prudent use of antibiotics not only in humans but also in animals.

Keywords: Antibiotic Resistance, *E. coli*, Qnrs, Water Sources.

THE PREVALENCE OF ESBLS PRODUCING PSEUDOMONAS AERUGINOSA ISOLATED FROM CLINICAL SAMPLES OF KHORRAMABAD CITY

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Background: *Pseudomonas aeruginosa* is among the most important nosocomial bacterial infections with innate resistance to many antibiotics. Broad-spectrum beta-lactamase enzymes areknown as an important cause of the emergence of *Pseudomonas aeruginosa* drug-resistant isolates. Resistance due to the production of these enzymes in Pseudomonas is rising. These studies have been conducted to find out the prevalence of ESBLs producing enzymes in the Pseudomonas aeruginos in Khorramabad city.
Methods: 70 cases of *Pseudomonas aeruginosa* were isolated from different samples of patients within a year in Treatment Centers Khorramabad city. Then the isolates were detected by routine biochemical tests were. Antibiotic susceptibility of isolates was assessed using Double Disk Diffusion method. ESBLs producing phenotype among isolated tested using the disc cefotaxime, ceftazidime alone and in combination with Clavulanic acid (Combined Disk Test) was conducted.
Results: Combined disk test results showed that among 70 *Pseudomonas aeruginosa* isolates, 25 of them were equal to 35.7% of the phenotype of beta-lactamase-producing enzymes.
Conclusion: According to the increasing incidence of beta-lactamase-producing strains of broad-spectrum, appropriate treatment protocols based on the antibiogram pattern strains, are highly recommended. This is a serious alarm for the use of infection control measures in order to prevent further spread of this infection.

Keywords: *Pseudomonas aeruginosa*, Esbls, Double Disk Diffusion

MOLECULAR ANALYSIS OF CLINICAL SPECIMENS OF CARBAPENEM RESISTANCE IN GRAM NEGATIVE BACILLI

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Background: In this study, while locally carbapenemase resistance surveillance is done, it is also aimed to epidemiological database of Turkey.
Methods: In period of one year between February 2012 and March 2013, 168 carbapenem-resistant Enterobacteriaceae members collected from several hospitals, clinics and polyclinics of Cukurova University, Başkent University, and Faculty of Medicine of Mustafa Kemal University. The confirmed samples,antibiotic susceptibility tests phenotypically and the PCR and sequence analysis method genotypically were investigated for carbapenemase enzymes.
Results: Among consisting strains of this study, resistance to over 80% of Penicillin, beta-lactam/beta lactamase inhibitor combinations, cephalosporins, ertapenem, imipenem and trimethoprim / sulfamethoxazole, 50-70% resistance to aztreonam, meropenem, gentamicin, tetracycline, fluoroquinolone type antibiotics, resistance to colistin reached level 5.2% was determinded.Among resistant strains of the most common carbapenemases, there are such as the OX-48 and OXA58 group D carbapenemases which their nonfermentative microorganisms seen in GES and PR enzyme and some *P.aeruginosa* and Enterobacteriaceae species seen in the IMP, VIM, NDM of enzymes were observed. In our country, sequencing strains expressing of VIM-7 enzyme analysis method have been first described in this study.
Conclusion: Carbapenemase gene diversity of our region in resistant strains is similar to the global diversity. However, wider region and a plurality of strain containing researches must be conducted.

Keywords: Carbapenemase,Antibiotic Resistance,Beta Lactamase

DETECTION OF IMIPENEM RESISTANCE DUE TO PRODUCTION OF METALLO-BETA-LACTAMASES IN ENTEROBACTERIACEAE ISOLATED FROM CLINICAL SPECIMENS BY PHENOTYPING AND GENOTYPING METHODS

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Background: Since the incidence of infections caused by MBLs producing Enterobacteriaceae is increasing worldwide, the aim of this descriptive study was the detection of MBLs producing in Enterobacteriaceae strains isolated from clinical specimens in Yazd teaching Hospitals using PCR method.
Methods: This research was performed on Enterobacteriaceae strains collected from various specimens of in-patients in three teaching hospitals dependent to Shahid Sadoughi university medical sciences from 2011.2.1 until 2012.2.19 in Yazd. All isolates were identified by conventional methods. The antimicrobial susceptibility test was performed by Kirby-Bauer method according to CLSI protocols. MBL production was detected by Hodge test. PCR method using specific primers for blaIMP-1, blaVIM-1 and blaNDM was performed.
Results: According to the results, of the 100 Enterobacteriaceae isolated, 26% were resistant to imipenem. Evaluation of Hodge test showed that 7 strains (7%) had Metalo-beta-Lactamases enzyme. 6 of these Metallo-beta-lactamases producing strains was isolated from urine culture and one Metallo-beta-lactamases producing strain was isolated from blood culture. Evaluation of blaIMP-1, blaVIM-1 blaNDM genes showed that among the Metallo-beta-Lactamases producing Enterobacteriaceae strains, 5 *E. coli* and one *Enterobactercloacae* strains had blaIMP-1 gene and one *Klebsiella* strain had blaVIM-1 gene. None of them had blaNDM gene. All MBL producing isolates were multi drug resistant.
Conclusion: The results show the high frequency of imipenem resistance in Enterobacteriaceae strains. In addition, the results show high prevalence of blaIMP-1 gene in MBLs producing Enterobacteriaceae.

Keywords: Enterobacteriaceae, Metallo-Beta-Lactamases, IMP, VIM, PCR

MOLECULAR STUDY OF OXA AND KPC TYPES' B-LACTAMAESES ENZYMES IN ESCHERICHIA COLI AND KLEBSIELLA PNEUMONIAE ISOLATES COLLECTED FROM IMAM REZA HOSPITAL, TABRIZ

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Background: The most important resistance mechanism against β- lactam antibiotics is production of β-lactamase enzymes among Gram-negative bacteria. By the way, the evaluation of clinical isolates in suspect of extended- spectrum β- lactamases producing is not performedroutinely in laboratories. The aim of this study was to determine antibiotic resistance patterns in *E. coli* and *K. pneumoniae* by ESBLs-producing isolates.
Methods: During one year, 82 *E. coli* and 18 *K. pneumoniae* isolates were collected from Imam Reza Hospital in Tabriz, Iran. Different phenotypic methods like combined disk and Modified Hodge Test were applied for production of ESBLs as recommended by the guide lines of the CLSI. Then detection of blaOXA and blaKPC genes was investigated in the isolates by Polymerase Chain Reaction (PCR) Method.
Results: From the isolates tested, 55(67.07%) *E. coli* and 4(22.22%) *K. pneumoniae* produced ESBL by combined disk method. 3(3.7%) *E. coli* isolates and 8(44.4%) *K. pneumoniae* isolates produced carbapenemases by Modified Hodge Test. From 82 *E. coli* isolates tested, 11(13.4%) and 15(18.3%) had blaOXA and blaKPC genes, respectively. In 18 *K.pneumoniae*isolates tested, 10(55.6%) had blaOXA and blaKPC genes, respectively.
Conclusion: The present study showed that *E. coli* and *K. pneumoniae* isolates producing β-lactamase enzymes are increasing. There are some similarities and differences in the antibiotic resistance patternsand ESBL production among the isolates in different areas of Iran and other countries. It seem to a combined of molecular methods and phenotype methods is necessary for complete detection of Beta- lactamases.

Keywords: *Escherichia coli, Klebsiella pneumoniae*, Extended Spectrum B- Lactamase, PCR, Blaoxa And Blakpc

EVALUATION OF ANTIBACTERIAL PROPERTIES OF CDSE NANOPARTICLES AGAINST SOME PATHOGENIC BACTERIA

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Background: Chemical precipitation method was used in order to synthesize of CdSe quantum dots (2 -3nm). Their physical properties and characteristics were assessed by X-ray diffraction, ultraviolet-visible spectrophotometry and scanning tunneling microscopy and it was shown that the obtained CdSe quantum dots have a cubic crystal structure.It was also shown that size and optical properties of CdSe quantum dots are related to the temperature (The temperature used by UV-Vis photometer).
Methods: Antibacterial effects of CdSe nanoparticles against some pathogenic bacteria were investigated. *Pseudomonas aeruginosa*, Actinomycet and *Salmonella typhi* were used as microorganisms tested. Disc bacteriological tests were used in order to assess the antibacterial effects of CdSe. The concentrations from 0.3 to 20 mg/mL of CdSe were investigated.
Results: It was concluded that the zone of inhibition diameter was strongly and directly related to the CdSe concentration. Actinomycet was the most affected bacteria.
Conclusion: The antibacterial activity of CdSe nanoparticles was assessed by the disc and well diffusion agar methods. By increasing the nanoparticle concentration in wells and discs, the growth inhibition and diameter of inhibition zone have also been increased. The sizes of inhibition zone were different according to the type of bacteria and the concentrations of CdSe QDs, the maximum diameter was observed for Actinomycet.

Keywords: Quantum Dots, *Pseudomonas aeruginosa*, Actinomycetes, *Salmonella typhi*

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EMERGENCE OF VANCOMYCIN RESISTANT STAPHYLOCOCCUS AUREUS (VRSA) IN MIDDLE EAST

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Background: *Staphylococcus aureus* is one of the most important pathogens worldwide. The emergence of Methicillin-Resistant *Staphylococcus aureus* (MRSA) made the vancomycin as the only therapeutic choice. Unfortunately, due to excessive use of vancomycin, decreased susceptibility and increasing resistance against this antibiotic has been reported worldwide. Here we studied the emergence vancomycin Resistant *Staphylococcus aureus* (VRSA) in different regions of Middle East.
Methods: All related data bases were screened for articles and abstracts reporting Vancomycin-Resistant *Staphylococcus aureus* in Middle East by related keywords. Appropriate phenotypic and genotypic studies were included in this review.
Results: Reviewing all articles/abstracts revealed 43 reports of vancomycin-Resistant *Staphylococcus aureus* (VRSA) isolates from Middle East. Most of the studies did not fulfill the Clinical and Laboratory Standards Institute (CLSI) Criteria on Vancomycin-Resistant *Staphylococcus aureus* (VRSA) identification. Until 2012, 3 VRSA strains were reported from Iran and one from Pakistan. During 2012 to 2014, there was one additional report from Iran. Also in this duration, two new strains from Pakistan and Egypt were reported.
Conclusion: Vancomycin-Resistant *Staphylococcus aureus* (VRSA) is a global health threat. According to the expanding reports on emersion of Vancomycin-Resistant *Staphylococcus aureus* (VRSA), more attention should be paid to the proper prescription of this antibiotic as the only choice of life threating staphylococcus infections.

Keywords: *Staphylococcus aureus*, VRSA, Middle East

EVALUATION OF THE HIGH LEVEL RESISTANCE TO THE AMINOGLYCOSIDE IN ENTEROCOCCUS FAECALIS BY THE MULTIPLEX-PCR METHOD

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Background: High-level gentamicin resistance (MIC ≥ 500 mg/L) is commonly intermediated by the aac (6′)-Ie-aph (2″)-Ia gene, which codes the bifunctional enzyme AAC (6′)-APH (2″). In the recent years, three new aminoglycoside resistance genes [aph (2″)-Ib, aph (2″)-Ic and aph (2″)-Id] that also mediate resistance to gentamicin, have been detected in *enterococci*. Other genes such as the aph (3′)-IIIa and ant (4′)-Ia, that encode the APH (3′) and the ANT (4′) have also been identified.
Methods: In this study, a total of 100 samples were obtained from the various specimens, including; urine, wound, sputum, abscess and tissue. The grown colonies were initially identified by biochemical routine tests. Finally, Multiplex-PCR assay for aac(6′)-Ie -aph(2″)-Ia, aph(2″)-Ib, aph(2′)-Ic, aph(2′)-Id, aph(3′)-IIIa, ant(4′)-Ia genes amplification were done in order to confirm bacterial colonies as *Enterococcus faecalis*.
Results: In all, eighty four (84%) *Enterococcus faecalis* isolates were recovered from 100 specimens. The highest and lowest isolates were related to urine (48%) and sputum (2%), respectively. Antibiotic susceptibility test results showed that the highest and lowest resistance is related to the tetracycline and nitrofurantoin, respectively. The Multiplex PCR results showed the aac (6′)-Ie-aph (2″)-Ia, ant (4′)-Ia and aph (3?)-IIIa genes in the 6% isolated bacteria from the urine, 2% from the wound and 1% from the Pleural. Amplification of the aac (6′)-Ie-aph (2″)-Ia and aph (3′)-IIIa genes were positive in the 25% isolated strains from the urine, 3% from the wound and 2% from the Plural. 9% isolated strains from the urine, 3% from the wound and 1% from the Plural were showed aac (6′)-Ie-aph (2″)-Ia and ant (4′)-Ia genes in the M-PCR.
Conclusion: A correct diagnosis quickly and decisively, is useful to prevent increased resistance to antibiotics in the high level aminoglycoside resistance (HLAR) strains. For this aim, molecular methods such as Polymerase Chain Reaction (PCR) recommended.

Keywords: High Level Resistance, Aminoglycoside, *Enterococcus faecalis*, Multiplex-PCR

THE ANTIBIOTIC RESISTANCE OF KLEBSIELLA PNEUMONIAE STRAINS ISOLATED FROM URINARY TRACT INFECTIONS OF SHIRVAN CITY-NORTH KHORASAN IN 1392

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Background: Urinary tract infections are the most common infections in humans. *Klebsiella pneumoniae* is opportunistic pathogen in nosocomial infections. Improvement of Beta-Lactamase (ESBL) in *Klebsiella pneumoniae* strains lead to the development of antibiotic resistance and mortality in patients. The purpose of this study was to evaluate the antibiotic resistance in *Klebsiella pneumoniae* strains isolated from urinary tract infections in Imam Khomeini Hospital in Shirvan city-North Khorasan in 1392.
Methods: After isolation of *K. pneumoniae* strains from urine cultures, the antibiotic resistance of the isolates was investigated with conventional approaches using the disk diffusion method according to CLSI standards. The standard discs of ampicillin, gentamicin, cefotaxime, cephalexin, cephalothin, nitrofurantoin, ceftriaxone, Nalidixic acid were used.
Results: Through the total of 2584 cases, 255 urine samples (8.9%) were positive, in which 26 (10.1%) *Klebsiella pneumoniae* was isolated. Among the *Klebsiella pneumonia* isolates, 50% to ampicillin, 6.34% to nitrofurantoin, 7.30% to cephalothin, 2.19% to Nalidixic acid, 2.19% to cefotaxime, 3.15% to gentamicin, 3. 15% to ceftriaxone and 5.11% were resistant to cephalexin.
Conclusion: The antibiotic resistance of *Klebsiella pneumoniae* in Shirvan was similar to resistance pattern of other parts of the country. Moreover, the results showed that there is less resistance to cephalexin in the isolates.

Keywords: Antibiotic Resistance, *Klebsiella pneumonia*, Urinary Tract Infection

FREQUENCY OF EXTENDED-SPECTRUM BETA-LACTAMASE-PRODUCING KLEBSIELLA PNEUMONIAE ISOLATES FROM URINARY TRACT INFECTIONS IN TEACHING HOSPITAL IN SHAHREKORD BY PCR METHOD.

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Background: The aim of this study was to investigate the frequency of ESBL producing *Klebsiella pneumonia* at an educational hospital in shahrekord, Iran.
Methods: This study was conducted at Shahrekord University of medical science. Totally, 150 isolates of *Klebsiella pneumonia* bacteria were selected from out-patient of Hajar and kashani university hospitals. Uropathogens were identified through culture, microscopy and biochemical tests. To detect possible ESBL production, combined double disc synergy test was performed by disc of ceftazidime (30 mg) alone and in the presence of clavulanate (30 mg/10 mg) at a distance of 25 mm, on a Mueller–Hinton agar plate. Detection of SHV gene was examined in ESBL positive strains by PCR.
Results: Combined double disc synergy test was applied to detect ESBL in 75*Klebsiella pneumoniae* isolates that are resistant to ceftazidime using ceftazidime. Among the 48 ESBL-producing *K. pneumonia* strains, 18(37.5%) were identified as SHV producing strains.
Conclusion: The spread of ESBL-producing bacteria has been strikingly rapid worldwide, indicating that continuous monitoring systems and effective infection control measures are required. Therapeutic options for infections due to ESBL producers become increasingly limited. Molecular detection and identification of beta lactamases would be essential for a reliable epidemiological investigation of antimicrobial resistance. Therefore, ESBL producing organisms should be identified quickly so that appropriate antibiotic usage and infection control measures can be implemented.

Keywords: *Klebsiella pneumonia*, ESBL, SHV

PREVALENCE OF PLASMID-MEDIATED AMPC B-LACTAMASES: FIRST REPORT OF CMY-2-TYPE AMPC B-LACTAMASE RESISTANCE IN Iran

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Background: The aim of this study was to determine the prevalence of *K. pneumoniae* encoded bla CMY-2 gene, isolated from clinical specimens.
Methods: In this Analytic-descriptive cross-sectional study, 144 isolates of *Klebsiella* spp. were collected from the clinical specimens such as wounds (7cases), supra pubic (1 case), blood (8 cases), septum (2 cases), catheter tips (3 cases), urine (106 cases), CSF (1 case), skin lesion (1 case), tracheal (9 cases), anal (2 cases), throat (1 cases) and eye culture (3 case) from Rasht hospitals from February to August 2013. After identification of isolates by biochemical methods, the antibiotic susceptibility test (Kirby-Bauer method) was done according to CLSI guideline against 20 antibiotics. The combined disk method (Double disk) was then carried out for detection of ESBLs of *Klebsiella* spp. Among ESBL producers of *Klebsiella* spp. blaCMY-2 was detected by PCR using specific primers. Then, PCR products were subjected to electrophoresis on 1.5 % agarose gel. Finally, PCR products were confirmed by sequencing.
Results: Among the *Klebsiella* 144 clinical isolates, 57 (39.6%) isolates were ESBL producers. The most prevalent ESBL producers were isolated from urine sample (33.57). The most resistance in *Klebsiella* spp. were belong to Oxacillin and Amoxicillin (98.1% and 97.2%, respectively) and Imipenem was the most effective antibiotic (95.3%) against all isolates. Among 57 ESBL producers of *Klebsiella* spp. only 12 cases (21.01%) were contained blaCMY-2 gene. This is the first report of blaCMY-2 gene found in *Klebsiella* spp. in Iran.
Conclusion: Due to high frequency of ESBL producing *Klebsiella* spp. isolated from clinical specimens, antibiogram should be conducted to choose the best antibiotic against *Klebsiella* spp and empirical treatment should be avoided to reduce antibiotic resistance development.

Keywords: *Klebsiella* Spp., ESBL, Antibiotic Resistant, Blacmy-2

DETECTION OF BLASHV-1 GENE IN ESCHERICHIA COLI AND KLEBSIELLA SPP. ISOLATES IN RASHT HOSPITALS

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Background: The aim of this study was to determine the prevalence of blaSHV-1 gene in *E. coli* and *Klebsiella* strains isolated from hospitalized patients in Rasht, Iran.
Methods: A total of 246 clinical isolates of *Escherichia coli* and 82 *Klebsiella* spp. were isolated from clinical specimens (urine, blood, wound and etc.) of hospitalized patients from Rasht hospitals, during eight months. The antibiotic susceptibility test (Kirby-Bauer method) was performed according to CLSI standards against 20 antibiotics. Moreover, all isolated bacteria were screened for ESBL production by combined disk diffusion method. PCR was performed to detect blaSHV-1 gene in ESBL positive isolates. PCR products were confirmed by sequencing. Data were analyzed by SPSS ver 21.
Results: Among 246 *E. coli* and 82 *Klebsiella* isolates, 95 (38.6%) and 34 (41.5%) strains were ESBL producers, respectively. The most prevalent ESBL producers were isolated from urine samples in both Genera. In case of *E.coli*, the highest resistance rates were belongs to Oxacillin, Cephalotin and Ampicillin (100%, 97.9% and 100%, respectively). Also, Amoxicillin, Ampicillin and Cephalotin and Oxacillin had the most resistance rate to *Klebsiella* spp. (100%, 97.9% and 97.1%, respectively). However, most of strains were susceptible to Imipenem in both strains (100% and 85.3%, respectively). PCR results revealed that among *E. coli* and *Klebsiella* spp. ESBL producers, the prevalence of blaSHV-1 gene was 7.4% and 44.1%, respectively.
Conclusion: The frequency of ESBL producing strains among clinical isolates has been steadily increasing. Advance drug resistance surveillance and molecular characteristics of ESBL isolates is necessary to guide the appropriate and judicious antibiotic use.

Keywords: *E. coli, Klebsiella* Spp., ESBL, Antibiotic Resistance, Blashv-1

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EVALUATION OF THE ANTIMIROBIAL EFFECT OF LACTOBACILLUS GASSERI ON SALMONELLA ENTERICA SEROTYPE ENTERTIDIS

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Background: *LactoBacillus* spp., as probiotics bacteria, is being increasingly studied for their inhibitory activity against pathogenic bacteria. There is some evidence that they are not effective against gram –negative bacteria.The objective of this study was to investigate the antagonistic activity of *lactoBacillus gasseri* (Lga C009) against resistant *Salmonella enterica* serotype enteritidis (ATCC 13311).
Methods: In this study, Antimicrobial activity of common bacteria causing gastroenteritis in children (*Salmonella enterica* serotype entertidis ATCC13311) was evaluated using three microbiological methods, Disk Diffusion Agar, Well Diffusion Agar and Agar spot test.
Results: The highest inhibitory effect achieved by Well Diffusion Agar with an inhibitory zone diameter of 0.2 ±0.7 mm and the minimum belonged to Agar spot test with an inhibitory zone diameter of 0.3 ±0.18 mm (P <0.05). By comparison three methods mentioned above, the well diffusion agar method was shown more sensitive than the other methods.
Conclusion: Results showed that the using of lacto*Bacillus* bacteria in food could prevent of gastrointestinal infections and also, it can play a role in inhibitory effect on the common gastrointestinal microorganisms, particularly in elder persons and children that is an important approach and strategic for human health.

Keywords: *Lactobacillusgasseri*, *Salmonella*, Antimicrobial Activity

DETECTION OF BLACTX-M, BLATEM, BLASHV GENES IN KLEBSIELLA PNEUMONIAE STRAINS ISOLATED FROM TWO HOSPITALS OF TEHRAN, Iran

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Background: An increasing clinical incidence of antibiotic-resistant *Klebsiella pneumoniae* is a major global health care issue. Therefore, the aim of this study was detection of blaCTX-M, blaTEM, blaSHV genes from two hospitals of Tehran, Iran.
Methods: This study was done on 83 *Klebsiella pneumoniae* isolated from Mofid Children and Taleghani hospitals in Tehran, Iran. Antibiotic susceptibility tests were performed by Kirby-Bauer disc diffusion and Broth Microdilution methods according to CLSI guidelines. The blaCTX-M, blaTEM and blaSHV Plasmid genes were detected by PCR and sequencing methods.
Results: From 83 *Klebsiella pneumoniae* strains, 48 (57.8%) were ESBL positive. The existence of blaTEM, blaSHV and blaCTX-M genes were detected in 24 (50%), 30 (62.5%) and 28(58.33%) of ESBL-producing isolates, respectively. In this study, Fosfomycin and Tigecycline were more active than other antibiotics. The nucleotide sequence data reported in this paper have been submitted to the GenBank sequence database and assigned under accession number KF513160 for blaCTX-M-15.
Conclusion: The prevalence of beta-lactamases-producing *Klebsiella pneumoniae* detected in this study is of great concern, which requires infection control measures including antibacterial management and identification of beta-lactamases-producing isolates.

Keywords: *Klebsiella pneumoniae*, B-Lactamases, Blactx-M, Blatem, Blashv

THE PREVALENCE OF TEM GENE AMONG EXTENDED-SPECTRUM BETA-LACTAMASES PRODUCING ESCHERICHIA COLI IN KHORRAM ABAD, Iran

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Background: Extended-spectrum beta-lactamases (ESBLs) producing *Escherichia coli* (*E. coli*) cause many serious infections including urinary tract infections, gastroenteritis, and neonatal meningitis. The aim of this study was to determine the prevalence of TEM gene among ESBLs Producing *E. coli* isolated from clinical samples of patients attending to selected hospitals in Khorram Abad, Iran.
Methods: In this study, during the one-year period in 2012, 150 strains of *E. coli* were isolated and identified by routine tests. ESBLs productionwas determined using a combination of clinical phenotype, including disk and disk diffusion agar by CLSI standardized. The prevalence of TEM gene was determined by PCR method.
Results: Among the 150 *E. coli*, 68 (45.33%) strains producing ESBLs were isolated using phenotypic tests. The frequency of TEM gene among the ESBLs producing isolates was 45 (66.17%) strains.
Conclusion: The results of this study showed that the prevalence of TEM gene among ESBLs producing *E. coli*was high. It seems that continuous surveillance is essential to monitor the ESBLs producing microorganisms in hospitals and community.

Keywords: *Escherichia coli,* Extended Spectrum Beta-Lactamase (ESBL), TEM

THE FIRST REPORT OF THE QNR, AAC(6')-IB-CR AND QEPA GENES IN QUINOLONE RESISTANT ESCHERICHIA COLI AND KLEBSIELLAPNEUMONIAE IN Iran

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Background: This study therefore was designed to characterize the prevalence of plasmid-mediated quinolone resistance (PMQR) determinants qnr, aac(6)-Ib-cr and qepA among quinolone resistant of *Escherichia coli* and *Klebsiella pneumoniae.*
Methods: One hundred and thirty-four quinolone resistance isolates were collected during nine months. ESBL production was determined by the Clinical and Laboratory Standards Institute (CLSI) ESBL confirmatory test. MICs of two antimicrobial agents were determined by E-test and screening for the qnrA, qnrB, qnrS, aac(6)-Ib-cr and qep A genes was carried out by PCR amplification.
Results: Of 134 quinolone resistnce isolates, 69.4% (n= 93) were able to produce ESBL (66.1% of *E. coli* and 73% of *K.pneumoniae*). The PMQR gene *qnr*A, qnrB and qnrS were found in 5.3% (n= 5), 14.9% (n= 14) and 13.8% (n= 13) of isolates respectively. Most of the qnr positive strains were ESBL producers. Sixty-six (49.3%) isolates were positive for aac(6)-Ib -cr that 54.5% of them were *E. coli* and 45.5% were K.pneumoniae. Twenty (14.9%) of isolates were positive for *qep*A.
Conclusion: This study highlights the prevalence of quinolone resistance determinants qnr, aac(6)-Ib-cr and qep A associated with ESBL production and multidrug- resistant *Escherichia coli* and *Klebsiella pneumonia*. This is the first report of the plasmid mediated fluoroquinolone efflux pump, qepA and aac(6)-Ib-cr and it is also the first report of PMQR screening in *K.pneumoniae* isolates in Iran.

Keywords: *K.pneumoniae*, *E.coli*, Plasmid Mediated Quinolone Resistance, Aac(6)-Ib-Cr, Qep A

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THE STUDY OF THE ANTIBIOTIC RESISTANCE PATTERN OF PSEUDOMONAS AEROGINOSA STRAINS ISOLATED OF CLINICAL LABORATORY FROM MARKAZI PROVINCE -Iran

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Background: The aim of this study was to determine resistance to anti-pseudomonal antibiotics in *Pseudomonas aeruginosa* strains.
Methods: 55 isolates of *P. aeruginosa* from different clinical specimens of clinical laboratory of Markazi province were isolated and identified through microbiological methods, including Gram staining, oxidase, Indol, and oxidative-fermentative tests. Antibacterial susceptibility test for imipenem, meropenem, amikacin, gentamicin, ciprofloxacin, levofloxacin, aztreonam, cefepime, piperacillin, piperacillin/tazobactam and ceftazidime was performed using disk diffusion (Kirby-Bauer) method.
Results: Of 55 isolates, 40 (72.7%) were MDR and 21 (38.1%) were PDR. 62.8% of the isolates were resistant to ceftazidime and 66.7% to piperacillin.The lowest rate of resistance was related to amikacin (25%) and gentamicin (20%).
Conclusion: The results of this study indicated a high rate of antibiotics resistant of *Pseudomonas aeroginosa* strains to different antibiotics. The results suggest that antibiotic resistance can be determined by choosing the appropriate drug to treat patients.

Keywords: Antibiotic, *Pesudomonas aeruginosa*,Multidrug Resistance, Pan-Drug Resistance

PREVALENCE OF EXTENDED SPECTRUM BETA-LACTAMASE IN KLEBSIELLA PNEUMONIAE ISOLATES COLLECTED FROM PATIENT ATTENDING ZAHEDAN TEACHING HOSPITALS

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Background: This study was conducted to determine the prevalence of *Klebsiella* resistant to cephalosporins and the prevalence of those producing ESBL.
Methods: A total of 100 clinical isolates of *Klebsiella* were tested by the disk diffusion method according to Kirby-Bauer to determine their antibiotic susceptibility and by the double-disk synergy method to detect the presence of ESBL.
Results: The results show that 81% of *Klebsiella* isolates tested were resistant to cefixime, 80% for cefotaxime, 48% for ceftazidime, 74% for ceftriaxone and 60% to aztreonam. Testing for ESBL revealed that the prevalence of ESBL producers in clinical *Klebsiella* isolates ranged from 63% to 68%.
Conclusion: We found that 68 of *Klebsiella* spp isolates were ESBL producers. There is a need to carefully formulate therapeutic strategies to control infections in teaching Hospitals. The high percentage of drug resistance in ESBL producing *Klebsiella* spp suggests that routine detection of ESBL is required by reliable laboratory methods.

Keywords: Extended Spectrum Beta-Lactamase, *Klebsiella*, Antibiotics

DETECTION OF AMPC BETA-LACTAMASE AMONG ENTEROBACTER SPP. ISOLATED FROM HOSPITALS IN TEHRAN AND QAZVIN, Iran

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Background: The aim of this study was to determine the prevalence inducible AmpC beta-lactamases by the phenotypic method.
Methods: A total of 95 *Enterobacter* isolates were collected from Tehran and Qazvin hospitals. After identification, antibiotic-resistant were studied by antibiotic susceptibility testing methods, Then the presence of inducible-AmpC identified based on the disk approximation (D-test) assay using cefoxitin and ceftazidime.
Results: The results of the antibiotic susceptibility showed of the 95 isolates, 81(85.2%) isolates were as non-susceptible isolates to cefoxitin. AmpC inducibility is detected because of blunting of ceftazidime zone adjacent to the cefoxitin disc, that 22 isolates (23.15%) were positive in D-test.
Conclusion: It appears that, resistance of *Enterobacter* spp.,to beta-lactam antibiotics is frequently due to indiscriminate use of them. Detection of organisms producing these enzymes is difficult in clinical laboratory. The capability to identify AmpC is important to improve the clinical management of infections.

Keywords: *Enterobacter* Spp., Ampc Beta-Lactamase, D-Test

ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF ESCHERICHIA COLI CAUSING URINARY TRACT INFECTIONS IN TEHRAN, Iran

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Background: Uropathogenic *Escherichiacoli* (UPEC) are among the most common agents of urinary tract infection (UTI) worldwide. UPEC resistance to commonly used antibiotics represents a major health problem all over the world. Antimicrobial susceptibility testing provides information that allows physicians to select the most appropriate antimicrobial agents for treating a specific infection. This study aimed to assess the Antimicrobial susceptibility pattern of *Escherichia coli* causing urinary tract infections in Tehran, Iran.
Methods: A total of 103 UPEC isolates were collected from in and out-patients attending various hospitals in Tehran between March 2013 and February 2014. The samples were cultured on LB Broth media and the bacterial isolates were tested against antibiotics Trimethoprim-sulfamethoxazole, Nitrofurantoin, Nalidixic acid, Gentamycin, Cephalexin, Norfloxacin, Ceftazidime, Amikacin, Ofloxacin, Ceftriaxone and Ticarcillin using Kirby Bauer disk diffusion method according to the CLSI.
Results: Up to 65% of the isolates were resistant to Trimethoprim-sulfamethoxazole and about 90% of the isolates were susceptible to Ticarcillin, Amikacin and Ofloxacin; and 80% of the isolates were susceptible to Cephalexin, Nalidixic acid, Gentamycin, Norfloxacin, Ceftazidime, Ceftriaxone and Nitrofurantoin.
Conclusion: The obtained results suggest that antibiotic selection for empirical treatment should be based on individual drug-sensitive test results. There is also an urgent need to develop a new combination of chemotherapeutic agents and awareness on antibiotic use should only be issued when prescribed by physicians for the effective UTI management in hospitals.

Keywords: Urinary Tract Infection, Resistance, *Escherichiacoli*, Antibiotics

PRESENCE AND ANTIBIOTIC RESISTANCE PATTERNS OF STREPTOCOCCUS AGALACTIAE AND STAPHYLOCOCCUS AUREUS ISOLATED FROM BOVINE MASTITIS

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Background: Bovine mastitis is recognized as one of the most important diseases affecting the dairy industry. The antibiotic resistance test is important to achieve accurate treatment.
Methods: This study was carried out to determine the prevalence of *Streptococcus agalactiae* and *Staphylococcusaureus* in 800 bovine milk samples collected from different dairy farms located in the Meshkinshar region of Iran.
Results: A total of 256 bacterial pathogens were isolated, of which 56 isolates were identified as *S. agalactiae* and 32 isolates were identified as *S. aureus*. Antibiotic susceptibilities of the isolates were investigated by agar disk diffusion method.
Conclusion: All of the *S. aureus* isolates were resistant to ceftiofur and all of the *S. agalactiae* isolates were resistant to streptomycin. Sensitivity to other antibiotics tested was varied.

Keywords: *Streptococcus agalactiae*, *Staphylococcus aureus*, Bovine Mastitis

DETECTION OF BETA-LACTAMASE RESISTANCE GENES (BLANDM, BLAVIM) AMONG STRAINS OF ACINETOBACTERBAUMANNII ISOLATED FROM CLINICAL SAMPLES BY PCR

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Background: Nowadays, one of the drugs that have been used extensively throughout the world to treat multidrug resistant *Acinetobacter* infections is β-lactam family. *Acinetobacter* spp. produces β- lactamase enzymes that leads to inactivation of antibiotics and also develops resistance to this class of antibiotics. β-lactamases consist of different types that blaVIM and blaNDM are among the most prominent of them. The aim of this study was detection of the genes encoding these enzymes by PCR method in Tehran's hospitals.
Methods: This study was carried out on 100 samples of *A.baumannii*isolated from patients hospitalized in different hospitals of Tehran. The susceptibility tests were carried out according to the Clinical and Laboratory Standards Institute (CLSI) guidelines using disk diffusion method. DNA was extracted by boiling and then PCR method was done using designed primers (blaVIM, and blaNDM).
Results: Isolates of *A.baumannii* revealed the highest resistance to tobramycin, Amikacin, Ceftazidime, Cefepime, Ceftriaxone, Piperacillin, Trimethoprim, and Ampicillin. Sulbactam and Imipenem considered as effective drugs in this study. The result of the PCR method showed that 12 of the isolates (12%) contained blaNDM and 18(18%) possessed blaVIM genes.
Conclusion: These two genes are increasing among *Acenitobacter* spp. Therefore, identification of them by PCR method is important and necessary for preventing infections.

Keywords: Multidrug Resistant, A.baumannii, Blavim, Blandm, PCR

DUPLEX-PCR FOR DETECTION OF AMINOGLYCOSIDE RESISTANCE GENES IN ENTEROCOCCI

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Background: The objective of this study was identification and determinationof antibiotic resistance of isolated clinical *Enterococcus* by Duplex-PCR method in Tehran's Hospitals.
Methods: In during 2013-2014, this study was carried out on a group of patient (n=350) in Baqiyatallah, Milad, and Emam khomeini Hospitals. These bacteria were identified by biochemical tests and specific culture. Their resistance to different antibiotics was detected by the disk diffusion test according to CLSI(2013). Using primers (aac(6')-Ie-aph(2")-Ia and aph(3')-IIIa), a Duplex-PCR method was designed for identification of resistant *Enterococci* spp.
Results: According to the screening test, from 150 investigated *Enterococcus*, 77% belonged to the *E. feacalis* species and 23% belonged to the *E. feacium* species. The highest resistance was observed for Gentamicin and Erythromycin, the lowest resistance was observed for Teicoplanin and Linezolid. Sixty-three of isolates (42%) containedaac (6')-Ie-aph(2")-Ia gene and twenty-one (14%) possessed aph(3')-IIIa gene. It should be noted that 13 isolates (8.6%) were positive for both genes simultaneously.
Conclusion: The present study showed that the two genes conferred resistance to aminoglycosides, particular, Gentamicin and Streptomycin in *Enterococci* spp. The resistance is increasing in Tehran's hospitals, thus they must be determined rapidly by molecular methods such as Duplex-PCR. This is the way for preventing infections in Tehran's hospitals.

Keywords: Aminoglycoside Resistance, Duplex-PCR.

A STUDY ON ANTIBIOTIC RESISTANCE PATTERNS OF MAIN BACTERIA ISOLATED FROM SURGICAL ROOMS, INTENSIVE CARE UNITS (ICU AND NICU) AND BURN WARDS IN HAMADAN EDUCATION HOSPITALS

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Background: The aim of this study was the evaluation of antibiotic resistance patterns of main bacteria isolated from surgical rooms, Intensive Care Units (ICU and NICU) and Burn wards in Hamadan Education Hospitals
Methods: This was a cross-sectional study that 250 samples were randomly collected from environment and apparatus of intensive care units. The samples were inoculated in EMB and Blood agar using wet swabs and transferred to medical laboratory for identification. Strains were selected and cultured on Muler Hinton agar for antibiogram tests by CLSI method. The antibiotics disks were consisted of ampicillin, ceftriaxone, ceftizoxime, erythromycin, vancomycin, gentamicin, cephalexine, gentamycin, cefepim, azytromycin, imipenem and ciprofloxacin. Data was gathered through a questionnaire and analyzed using SPSS 16 software.
Results: The average rate of bacterial contamination of surgical rooms, Intensive Care Units (ICU and NICU) and Burn wards was 53%. Most bacteria isolated were as follow: Staphylococcus epidemidis (20.26%), *E. coli* (19.60%) and *Acinetobacter baumannii*(15.03%). The most contaminated places were enkubator of Fatemie NICU. Most of isolates (60%-90%) were sensitive against imipenem, vancomycin and ciprofloxacin, whereas most of them were resistant to ampicillin, erythromycin and gentamicin.
Conclusion: Our results showed the considerable bacterial contamination (53%) of Surgical rooms, Intensive Care Units (ICU and NICU) and Burn wards in particular with *Acinetobacter baumannii* and the high drug resistance in strains isolated from hospitals, it seems that sterilization and disinfection methods in hospitals were not performed correctly. Therefore it is recommended that health workers should be trained regularly to control the incidence of nosocmial bacterial.

Keywords: Nosocomial Infection, Antibiotic Resistance, Bacteria, Antibiotic

PREVALENCE OF CTX-M-15 TYPE BETA-LACTAMASE GENES IN ESCHERICHIA COLI STRAINS USING PCR METHOD

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Background: Extended spectrum B-lactamase (ESBL) genes play an important role in spreading B-lactam antibiotic resistance in the producing strains expressing these enzymes. The resistance of gram-negative bacteria, such as *Escherichia coli,* to different antimicrobial agents, has been increasingly reported. This study was conducted to determine the prevalence of ESBL in *Escherichia coli* isolates.
Methods: In this cross-sectional study, between March 2013 and February 2014, a total of 120 strains of *Escherichia coli* were isolated from clinical patient specimens ingeneral Hospital. Then, the frequency of ESBL- producing strains was determined by the combined disk method. The presence of the β lactamase gene CTX-M-15 in ESBL was assessed by PCR method.
Results: Atotal of 120 bacteria were isolated from urinary tract infections that*E. coli* 98 bacteria were *E. coli* and 76.4% of them were ESBL.55 cases were multi-drug resistance.18% of the strains were resistant to beta-lactamase broad CTX-M-15 type
Conclusion: Regarding to the increased rate of ESBLs producing strains, it is strongly recommended that the appropriate treatment protocol based on the antibiogram pattern of the strains can be used.

Keywords: Spectrum ß-Lactamases, *Escherichia coli,* CTX-M-15

FREQUENCY OF METHICILLIN-RESISTANCE AMONG CLINICAL ISOLATES OF STAPHYLOCOCCUS AUREUS BY PHENOTYPIC AND MOLECULAR METHODS IN YAZD CITY

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Background: The aim of this study was the survey of frequency of methicillin-resistance among clinical isolates of *S.aureus* by phenotypic and PCR methods in Yazd, Iran.
Methods: In this descriptive-cross sectional study, a total of 114 *S. aureus* isolates were collected from different specimens of patients admitted to Shahid Sadughi hospital in Yazd, Iran. Antimicrobial susceptibility testing was determined by disk diffusion method (Kirby-Bauer) and minimum inhibitory concentration of oxacillin (MIC) was performed by E.test method. PCR method was performed for detection of the mecA gene using specific primers.
Results: Out of 114 *S.aureus* strains, 54strains (47.4%) were isolated from wound, 23strains (20.2%) from tracheal aspirates, 18 (15.8%) from blood and 12(10.5%) from urine. Susceptibility testing by disk diffusion method was showed that 43 (37.7%) and 49 samples (43%) were resistant to oxacillin and cefoxitin respectively. MIC results showed that 46 (40.5%) samples were resistant to oxacillin, of which 27 (23.7%) cases were high-level resistant to oxacillin (MIC greater than 256 mg). While out of 114 strains, 54 samples (47.4%) were found to carry the mecA gene using PCR. Highest resistances to antibiotics were for penicillin (98.2%), ampicillin (99.1 %), tetracycline (55.3%), erythromycin (37.7%), clindamycin (32.5%), ciprofloxacin (32.5%), ofloxacin (31.6%), gentamicin (26.3%), respectively.
Conclusion: According to results of this study, the multidrug resistance (MDR) is common among *S.aureus* isolates.

Keywords: *Staphylococcus aureus*, MRSA, Meca, PCR

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ANTIBACTERIAL ACTIVITY AND CHEMICAL COMPOSITION OF MEDICINAL PLANT SATUREJA BAKHTIARICA BUNG AGAINST MULTI DRUG RESISTANT ACINETOBACTER BAUMANNI (ESBL)

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Background: Historically, plants have provided a source of inspiration for novel drug compounds. Plant derived medicines have made large contributions to human health and well being. Of late, there is an increment in the use of herbal products all over the world. Investigation of certain indigenous plants for their antimicrobial properties may yield useful compounds that may serve as lead molecules or novel chemical entities. Here, the chemical compositions and antibacterial activity of the essential oils obtained from *Satureja bakhtiarica* bung against multi drug resistant *Acinetobater baumanni* isolated from burned patients were evaluated.
Methods: Chemical compositions of essential oil were analyzed by gas chromatography mass spectrometry (GC-MS) method. Antibacterial activity of essential oil was evaluated by a well diffusion method. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined by the macrodilution method
Results: The GC-MS spectrums showed 13 compounds, in which the highest chemical composition was related to phenol (37.36%), thymol (22.65%) and p-cymen (19.29%) compounds. The essential oil of *Satureja bakhtiarica* bung showed good activity against tested bacteria, which is possibly due to the high levels of phenol in their compositions. The MIC and MBC values of *A.baumanni* sensitive to the essential oil were in the ranges of 3.12 to 6.25 respectively.
Conclusion: However, the essential oil of *Satureja bakhtiarica* bung is a suitable plant drug against multi drug resistant *A.baumanni.*

Keywords: Medicinal Plant, *Satureja Bakhtiarica* Bung, Antibacterial, A. *baumanni*

PHYTOCHEMICAL ANALYSIS, ANTIBACTERIAL ACTIVITY OF MARRUBIUM VULGARE L AGAINST ANTIBIOTIC RESISTANCE ESCHERICHIA COLI

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Background: The present study was carried out to determine the potential antibacterial effect of essential oil of *Marrubium vulgare* L. against *E.coli*, which is antibiotic resistant
Methods: All 12 strains of *E. coli* isolated from hospital inpatients in Zabol city were screened during the years 2011-2012. In this study, the essential oil of *Marrubium vulgare* L. obtained by hydrodistillation was analyzed by gas chromatography coupled to mass spectrometry (GC-MS) in order to determine their chemical composition. The minimum inhibitory concentrations were investigated to characterize the antimicrobial activities of this essential oil. The result show that thirty-one components were identified in the oil of *Marrubium vulgare.*
Results: The results demonstrated that the major components of the essential oil were Eudesmol (11%), Germacrene (10%), D-Citronelly formate (10%), Citronellol (8%), Geranyl tiglate (7.1%), and Geranyl formate (6.02%).
Conclusion: The least and highest MIC value of essential oil M. vulgare was 0.3 mg/ml and 5mg/ml, respectively.

Keywords: Antibacterial Activity, *Marrubium vulgare*, *E. coli*

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DISTRIBUTION OF VIRULENCE FACTORS IN UROPATHOGENIC ESCHERICHIA COLI ISOLATED IN KERMANSHAH

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Background: Uropathogenic *Escherichia coli* (UPEC) can cause UTI.For preventing itself against urine flow lavage, the bacteria have acquired a number of virulence factors, called Adhesins. These adhesins are expressed and controlled by different genes. The aim of this study was to determine some of the most important genes to control virulence factors of UPEC (pap, sfa and afa genes) coding the adhesins and phenotypic factors.
Methods: Totally 205 UPEC isolates were collected from UTI in and out patients. PCR was used to amplify genes. A drop of bacterial suspension, RBC and PBS were mixed For HA. Clump was considered as a positive test. To detect hemolysis, bacteria were grown on blood agar. Surface hydrophobicity was carried out by the SAT test.
Results: Frequency of pap, afa and sfa were 42 (20.5%), 17 (8.3%) and 44 (21.5%), respectively. Frequencies of haemaglutination, haemolysin and hydrophobicity were 138 (67.3%), 56 (27.3%) and 39 (19%), respectively. Among haemaglutination positive ones, 103 (74.6%) were mannose resistant. Our results highlight higher frequency of haemaglutination compared to other virulence factors, which can indicate a crucial role of this virulence factors in UPEC.
Conclusion: We concluded that major differences exist in the prevalence of virulence factors among different countries' UPEC. Association observed between pathogenicity and virulence factors may promote UPEC survival and growth within the urinary tract. Detecting these genes, as the main controller of UPEC virulence factors, may aid to better management of related infections.

Keywords: Uropathogenic *Escherichia coli,* Virulence Factors, Haemaglutination, Hydrophobicity, Hemolysin

MOLECULLAR TYPING OF ESBL PRODUCING UROPATHOGENIC ESCHERICHIA COLI IN WEST OF Iran

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Background: Urinary tract infection (UTI) is one of the most common bacterial infections in the world. Cephalosporin'sare commonly used for treatment of infections by *Escherichia coli* in UTI. However, resistance to these antibiotics is increasing in last decades. The aim of the study was to determine genotyping of ESBL producing UPECs in the west of Iran.
Methods: Totally,200 UPEC isolates from out-patients with UTI were collected in the community in west of Iran. Antimicrobial susceptibility and interpretation were performed by disk diffusion and CLSI guideline. Virulence factors for UPECs were screened by using PCR. UPECs were analyzed by PFGE (with the restriction enzyme XbaI) and banding patterns were analyzed by Phoretix1DPro software
Results: Out of 200 isolates of UPECs, 24.5% (n= 49) of isolates were positive for ESBL production. Resistance ranged from 0% for amikacin and imipenem to over 93.9% for carbnicillin and ampicillin. Frequencies of haemagglutination, haemolysin, and hydrophobicity were 25 (51%), 9 (18.3%), and 7 (14.28%), respectively. A total of 10 different types were obtained, including nine common clones and 1 individual clone.
Conclusion: We confirmed the prevalence of virulence phenotyping especially Haemagglutination among UPEC strains and that it can also contribute to virulence in these strains. Large Diversity in genotypes was observed in the isolates that could be indicative of different sources of infection in community acquired. The results of this study suggest that the risk of an outbreak in the future.

Keywords: Uropathogenic *Escherichia coli,* ESBL, Pulsed-Field Gel Electrophoresis, Virulence Factors

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VIRULENCE CHARACTERISTICS OF E.COLI ISOLATED FROM CHILDREN WITH URINARY TRACT INFECTION

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Background: Uropathogenic *Escherichia coli* (UPEC) is a causative agent in most urinary tract infections (UTIs), expressing a multitude of virulence factors. The aim of this study was to investigate some characteristics in UPEC isolates derived from urine specimens.
Methods: A total of 50 *E. coli* isolates were collected from patients suffering from UTI during September 2009 to September 2010.Biochemical and standard microbiological techniques were used to identify *E. coli* and then screened for phylogenetic typing groups, pathogenicity islands, hlyD and intI1 genes by polymerase chain reaction (PCR).
Results: We found a high number of PAI markers such as PAI ICFT073, PAI IICFT073, PAI I536, PAI IV536, PAI II J96, and PAI II536 significantly associated with UPEC. PCR phylogenetic typing groups revealing that the higher prevalence of uropathogenic strains were mainly found in subgroup B2 and D. High resistance to aztreonam, co-trimoxazole, cefpodoxime, and cefotaxime were found in UPEC isolates. It was shown that hlyD was present in 26% of UPEC, however, hemolysin was expressed as 42% ($p < 0.05$). The IntI1 gene was expressed as 24%.
Conclusion: Knowledge of the molecular details of uropathogenic *E. coli* is useful for development of successful strategies for treatment of urinary tract infection in human and complications associated with UTIs.

Keywords: *Escherichiacoli*, Virulence Characteristics, Antibiotic Susceptibility Pattern

PATHOTYPIC COMPARISON OF URINARY AND FECAL ESCHERICHIACOLI ISOLATED FROM CHILDREN WITH URINARY TRACT INFECTION

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Background: Uropathogenic *Escherichiacoli* (UPEC) is a causative agent in most urinary tract infections (UTIs), expressing a multitude of virulence factors. The aim of this study was to investigate the phylogenetic background and the presence of the hlyD and Integron I (intI1) genes in *E. coli* isolates derived from urinary and fecal specimens.
Methods: A total of 100 *E. coli* isolates were collected from patients suffering from Urinary Tract Infection (UTI) during September 2009 to September 2010 and screened for hlyD and intI1 genes by polymerase chain reaction (PCR).
Results: Phylogenetic analysis showed that *E. coli* is composed of four main phylogenetic groups (A, B1, B2 and D) and that Uropathogenic *E. coli* (UPEC) isolates mainly belong to groups B2 (54%) and D (34%) whereas groupA (44%) and D (26%) are predominant in Commensal *E. coli* isolates. It was shown that hlyD was present in 26% of UPEC and 2% of commensal *E. coli* isolates, however, hemolysin was expressed by 42% of UPEC and 6% of commensal *E. coli* isolates ($p < 0.05$). IntI1 gene was more frequently expressed in UPEC (24%) in comparison with commensal *E. coli* isolates (12%). High resistance to aztreonam, co-trimoxazole, cefpodoxime, and cefotaxime were found among UPEC isolates where as commensal *E. coli* strains were extremely resistant to co-trimoxazole, nalidixic acid and amoxicillin. A considerable difference between UPEC and commensal *E. coli* isolates was obsereved regarding to their phylogenetic groups, the presence of the integron class 1 and hly D gene, hemolysin activity and resistance pattern.
Conclusion: We concluded that the rate of multidrug to resistance tothe presence of class 1 integrons and hlyD gene were higher in UPEC isolates compared with *E. coli* strains. These findings will facilitate greater understanding commensal of the factors that contribute to the pathogenesis of UPEC

Keywords: *Escherichiacoli*, Urinary Tract Infection (UTI), Phylogenetic Typing Groups, Hlyd, Inti1

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GENOTYPING OF E.COLI ISOLATES FROM CHILDREN WITH URINARY TRACT INFECTION BY PULSE FIELD GEL ELECTROPHORESIS (PFGE)

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Background: Urinary tract infection is one of the most important diseases in children that delay of diagnosis and treatment may cause scar. *E. coli* because these infections in 75 % cases.Diarrheagenic *E. coli* have pathotypes but Pathotypes of *E. coli* which cause urinary tract infection have yet not determined. In this study we wanted to determine pathotypes uropathogenic *E. coli* by some virulence factors and genotyping methods.
Methods: Fifty urinary isolates from children with UTI were examined.Antibiotic resistant pattern was done by disk diffusion test or kirby bauer method. Some virulence factors such as adhesion group, protectins, and common toxins related to UPEC, intI1 class 1, micellaneous genes, pathogenicity islands were examined by the PCR method. Their genetic diversity studied by phylogenetic typing and by pulsed- field gel electrophoresis (PFGE).
Results: Some virulence factors were more prevalent in UPEC than fecal isolates as followed: PAI ICFT073, PAI IICFT073, PAI I536, PAI IV536, PAI II J96, PAIII536, gafD, focG, vat, usp, hlyD, sat, cnf1, picU, fliC(H7), kpsMTII, kpsMTIII. PCR phylogenetic group typing revealed that uropathogenic strains were mainly found in subgroups B2 and D.There were high diversity observed and no clonal dissemination was detected in urinary.
Conclusion: Our results gave new insights about Uropathogenic *E. coli* virulence factors. Therefore, being high diversity in our population caused, we couldn't uropathogenic pathotypes determination by genotyping and virulence factors detection. Knowledge of the molecular details of uropathogenic *E. coli* is useful for development of successful strategies for treatment of urinary tract infection in human and prophilaxy of complications associated with UTIs.

Keywords: Uropathogenic *E. coli,* Urinary Tract Infection, Virulence Factors, Genotyping

DETECTION AND INVESTIGATION OF ESCHERICHIA COLI IN CONTENTS OF DUODENUM, JEJUNUM, ILEUM AND CECUM OF BROILERS BY PCR

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Background: *Escherichia coli* strains cause a number of diseases in broiler chickens, ultimately leading to death or to a decrease in production or the condemning of carcasses. In this study, the isolation and molecular detection of *E. coli* from broiler chicken gut contents are described. Broilers were raised under conditions identical to those found in commercial broiler operations.
Methods: On 3, 15 and 35 days of age, 24 birds were randomly sacrificed and contents of duodenum, jejunum, ileum and cecum were removed. After DNA extraction, these samples were subjected to an optimized PCR to detect the presence of *E. coli* and to determine its presence or absence in intestinal segment contents at different ages.
Results: For all gut segments, a total of 41.7, 20.8 and 35.5% of samples were positive for *E. coli* by PCR on 3, 15 and 35 days, respectively. A total of 85.5, 25 and 85.5% cecum samples were positive for *E. coli* by PCR on 3, 15 and 35 days, respectively.
Conclusion: Posterior segments exhibited lower levels of *E. coli* compared with the anterior segments, especially the cecum. Furthermore the PCR protocol used in this work was shown to be an efficient method to detect *E. coli* in naturally contaminated intestinal samples, as accurate as and certainly quicker than culture and other detection methods.

Keywords: *Escherichia coli,* PCR, Broiler, Intestine

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Antibacterial Activity Of Terminalia Catappa Extract Against Escherichia Coli Isolated From Urinary Tract Infections

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Background: *Escherichia coli* (*E.coli*) are an important pathogen in the Urinary Tract Infection (UTI). *E. coli* is cause of 80% of UTI. Increasing antibiotic usage for *E. coli* infections has created antibiotic resistance. Subsequently, it is necessary to produce new antibiotics. Medical herbs with anti microbial activity have always been an important role in traditional medicine. The purpose of this study was to determine the antibacterial activity of methanol extract from fruit of *Terminalia catappa* against *E. coli* isolated from UTI and to compare with effects of selected antibiotics in vitro.
Methods: This research is a descriptive analytic study. First, a sample of methanolic extract from the fruit was prepared by maceration method. Then, its antibacterial activity against 124 isolates of *E. coli* from 150 samples of UTI was evaluated by well diffusion and then agar serial dilution method. Also, the MIC (Minimum Inhibitory Concentration) of extract was determined. The effect of selected antibiotics was tested by disk diffusion method.
Results: The frequency distribution tables, diagrams and T-test were used to describe and compare the results. The results demonstrated that the plant extract had been affected against 62 of *E.coli* strains(50%).The MIC of the extract for these bacteria was 20 mg/ml, while they were often sensitive to selected antibiotics(100% sensitive to Imipenem and 97% sensitive to Amikacin).There was significant differences between the effects of plant and antibiotics on *E.coli*($P<0.001$).
Conclusion: This study demonstrates that a methanolic extract of *Terminalia catappa* is no good effective on *E. coli* isolated from UTI and its effect is no better than that of selective antibiotics. Further investigations will be necessary.

Keywords: *Escherichia coli, Terminalia catappa*, Urinary Tract, Infection

PHYLOTYPING OF COMMENSAL ESCHERICHIA COLI ISOLATES FROM DAIRY COWS AND CALVES BY PCR

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Background: *Escherichia coli* is a commensal inhabitant of the intestinal tracts of healthy humans and many animal species, but it can also cause a wide range of diseases, ranging from diarrhea to extraintestinal infections. Four main phylogenetic groups, A, B1, B2, and D, constitute the bulk of the species.
Methods: In this study fecal samples were obtained from 126 animals including 74 dairy cows and 52 healthy calves. The samples were cultured on a biochemical medium for isolation of *E. coli*. *E. coli* were isolated from all of the samples. DNA were extracted by boiling and examined by multiplex PCRFrom each strain. Phylotyps of isolates were detected according to the presence or absence of chuA, yjaA genes and TSPE4.C2 genes.
Results: The 126 examined isolates were classified in three phylogenetic A, B1 and D groups and four phylogenetic subgroups A0, A1, D 1 and D2. None of the strains belonged to B2 group. Prevalence of A phylotype was 39.68% (50 isolates), B1 23.80 (30 isolates) and D 36.50% (46 isolates). According to phylogenetic results, subgroups were segregated in A0 and A1 25 isolates (19.84%) subgroups, 23 isolates (17.46%) in D1 and 24 isolates (19.40%) in D2 subgroup. The most prevalent phylotyps from dairy cows were A (39.18%), D (35.13%) and B1 (25.67%), respectively. In *E. coli* isolates from healthy calves, the most prevalent phylogroups was A (40.38%), D (38.46%) and B1 (21.15%).
Conclusion: In general, in farm animals, prevalence of A, B1 and D phylogroups are more than B2 groups, which may be the low resistance of isolates from B2 phylogroups against antibiotic usage play a role in this matter.

Keywords: *Escherichia coli,* Phylotype, Cow

THE STUDY OF COMBINED EFFECT OF MAGNESIUM OXIDE AND ZINC OXIDE NANOPARTICLES ON ESCHERICHIA COLI

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Background: Along with the rapid development of human life, Controling the harmful effects of microorganisms is inevitable. The purpose of this study wasantibacterial efficacy of both ZnO and MgO nanoparticles combined against microbial strains *Escherichia coli* was to determine whether the nanoparticles have a synergistic effect on each other or not.
Methods: This experimental study was conducted using *Escherichia coli* bacteria. At the frist time, MgO and ZnO nanoparticles on the antimicrobial effect of the combined suspension, cultured using a spot on the grass, and then studied the bacterial growth curve, and the broth was investigated. The suspensions of MgO and ZnO nanoparticles are also possible to use a combination of food safety and antimicrobial effect of the suspension on the antimicrobial activity of some antibiotics studied. Information obtained by Chi-square test using SPSS software is examined and the results were presented.
Results: Meadow medium contains no halo around the disc 0.25, 0.5, 0.75 of ZnO and 0.5, 0.5, 0.75 and MgO (ZnO 0.25+MgO 0.75), (ZnO 0.75+MgO 0.25), (ZnO 0/5+MgO 0/5) did not detect. Further, the amount of liquid cultures ZnO 0.5, MgO 0.5, ZnO 1.5, MgO 1.5, (ZnO 1.5+MgO 0.5), (ZnO 0.5+MgO 1,5) was repeated, and maximum reduction of 90% in relative growth rate (ZnO 0.5+MgO 1.5) were observed.
Conclusion: Magnesium oxide and zinc oxide nanoparticles have a synergistic effect on each other and they can be combined to reduce the number of bacteria used and Thereby lower concentrations is required than when nanoparticles are used by only.

Keywords: Biofilm, Microbiome, Nanoparticles

PREVALENCE OF SOME VIRULENCE GENES AMONG UROPATHOGENIC ESCHERICHIA COLI ISOLATES

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Background: *E. coli* strains isolated from urinary tract are known as uropathogenic *Escherichia coli* (UPEC), which cause 80-90% of community acquired urinary tract infection (UTI). UTI usually begins by colonization of pathogenic bacteria in bladder and subsequently the bacterial infection may ascend to kidneys. This study was conducted to investigate some of the virulence factors including fimH, hlyD and tsh, involving in UTI infection among UPEC isolates.
Methods: A total of 85 UPEC isolates from urine samples of outpatients were confirmed by conventional biochemical tests. PCR was done to detect fimH, hlyD and tsh genes using specific pairs of primers for each gene.
Results: PCR assays indicated that adhesins encoding gene, fimH was detected in 29 (34.1%) of the UPEC isolates while toxin encoding the gene, hlyD, and the gene encoding temperature sensitive hemagglutinin, tsh, were detected in 18 (21.2%) and 23 (27.1%) of these isolates, respectively. It is noteworthy to mention that 38 (44.7%) of these strains did not encode any discussed virulence genes.
Conclusion: Thehighest frequency of fimH among UPEC virulence genes reconfirms a crucial fimH crucial role in UPEC pathogenesis and suggests fimH inhibitors as backup therapeutics for UPEC associated UTI. However, high prevalence of isolates, which are not encoding fimH (75.9%), and relatively low frequency of isolates carrying other virulence genes suggest that further investigations are needed to be performed in order to clarify the role of other potential virulence factors in pathogenesis of these isolates.

Keywords: UPEC, Fimh, Hlyd, Tsh, PCR

THE OCCURRENCE OF CLASS 1 INTEGRON AMONG ESCHERICHIA COLI STRAINS ISOLATED FROM SURFACE WATER SOURCES IN ALBORZ PROVINCE

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Background: *E. coli* is considered as one of the most important causes of bacterial pathogenes transmitted via water and food. The aim of this study was to investigate the occurrence of *E. coli* strains carrying class 1 integron isolated from different water sources in Alborz province.
Methods: This study was carried out in 2013 in which all *E. coli* strains isolated from different water sources in Alborz province were subjected. *E. coli* strains were detected and identified using standard microbiological and biochemical tests. Then, the strains were subjected for presence of class 1 integron by PCR using specific primers targeting int1.The PCR amplicons were visualized after electrophoresis and stained with ethidium bromide.
Results: Ninety nine *E. coli* strains were isolated and included in the study. The PCR results showed that 26% of the strains harbored integron class 1 gene. Most of bacterial strains harboring int1 were multi drug resistant.
Conclusion: Our findings revealed high prevalence of int1 gene among *E. coli* strains circulating in surface water sources. This finding raises a concern about distribution of such threatening agents in these types of water sources and horizontal gene transfer between other waterborne bacterial species. Our results underline the need for enhanced laboratory capacity and coordinated surveillance strategies to control the further spread of these threatening agents.

Keywords: *E. coli*, Integron Class1, PCR, Water.

DISTRIBUTION OF FIMBRIAE VIRULENCE GENES IN DIFFERENT PHYLOGENETIC GROUPS UROPATHOGENIC ESCHERICHIA COLI STRAINS ISOLATED FROM PATIENTS WITH URINARY TRACT INFECTION IN SISTAN

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Background: Uropathogenic *E. coli* (UPEC) is the most important cause of urinary tract infections. These strains with specific virulence factors, including fimH, sfa, papC, foc and afaI have the potential pathogenicity in the urinary tract. Phylogenetic studies puts the bacteria into four main groups A, B1, B2 and D. Extra-intestinal pathogenic strains are substantially in B2 group, and less in D Group. The purpose of this study was to determine the prevalence of pathogenic genes, which are listed according to the type phylogroup *E. coli* isolated from patients with urinary tract infection in Sistan.
Methods: In this study, 100 *E. coli* samples were collected from patients with urinary tract infection in hospitalized patients and referring to Zahedan and Zabol laboratories. After the biochemical tests to confirm the samples, DNA was extracted by a boiling method. Prevalence of virulence genes using the PCR was performed. Triplex PCR was used for determining the phylogenetic groups.
Results: Prevalence of the fimH, sfa, papC, foc and afaI genes were determined, respectively, 95%, 81%, 57%, 16% and 12%., respectively. A, B1, B2 and D phylogenetic groups were 17%, 6%, 55% and 22%, respectively.These strains belonging to B2 group showed the highest presence of virulence genes.
Conclusion: This study indicated that strains belonging to B2 group are the most important and abundant phylogenetic groups among *E. coli* strains causing urinary tract infection

Keywords: Uropathogenic *E. coli* (UPEC), Urinary Tract Infections (UTI), Fimbriae

SURVEY OF ANTIBIOTIC-RESISTANCE PATTERNS IN E.COLI ISOLATED FROM PATIENTS WITH URINARY TRACT INFECTION IN RASHT

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Background: As the *E. coli* is the most common cause of urinary tract and nosocomial infections, the present study was designed to determine the antimicrobial susceptibility of *E. coli* in the urine culture of patients admitted to several Hospitals in Rasht.
Methods: In this descriptive case– series study, non- randomized purposive sampling was recruited for 195 urine positive sample. Antibiotic resistance profiles (Kirby- Bauer method) were performed according to the CLSI guideline against 19 different antibiotics. The combined disk method was used to detect ESBLs producers *E.coli*. All the data analyzed in SPSS version 19.
Results: *E. coli* strains were isolated from 76.93% women and 23.07% men. The majority of strains were isolated from children less than 10 years old. The most antibiotic resistance rates between Penicillins were belonging to Oxacillin, Ampicillin and Amoxicillin (100%, 85.64% and 83.58%), respectively. Among cephalosporins, Cephalotin and Cefixime were of the highest resistance rate (63.56% and 50.25% respectively) and Cefoxitin had the least resistance rate (11.79%). Among quinolones, the most resistance and susceptibility were belonging to Nalidixic¬ acid (85.12%) and to Ofloxacin (55.9%) respectively. Resistance to Nitrofurantoin, Tetracycline and Co-trimoxazol was also 8.71%, 75.9% and 63.07% respectively. All of the strains were susceptible to Imipenem. Gentamycin and Cefoxitin had the least resistance rate after Imipenem (8.2% and 11.79% respectively). Among 195 strains, 39.48% isolates were ESBL producers.
Conclusion: In this study, the highest susceptibility was observed for Imipenem. In clinical practice, it is also the first antibiotic prescribed to treat urinary tract infection. The highest resistance belongs to Oxacillin, exploring explains why this drug is not recommended to treat urinary tract infections.

Keywords: *Escherichia coli,* Antibiotic-Resistance, Urinary Tract Infection, ESBL.

DISTRIBUTION OF THE SUBTILASE CYTOTOXIN GENE (SUBA) AMONG SHIGA TOXIN-PRODUCING ESCHERICHIA COLI ISOLATED FROM DIFFERENT SOURCES

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Background: Subtilase cytotoxin is a potent AB5 toxin that first discovered in O113: H21 Shiga toxin-producing *Escherichia coli* (STEC) strain involving in an outbreak of hemolytic uremic syndrome (HUS) in Australia and introduced in 2004 as a novel bacterial toxin. It has been proposed that this toxin may augment the effects of Shiga toxins and may increase the severity of related diseases in humans. The aim of the present study was to investigate the occurrence of Subtilase encoding gene (subA) among STEC isolates from different sources in Iran.
Methods: For this purpose, a total number of 53 STEC strains that were isolated from cattle (35), sheep (3), pigeon (12) and humans (3) in recent years investigated using a specific polymerase chain reaction (PCR) assay for the subA gene. Then, all STEC isolates were screened for the Stx subtypes using a multiplex-PCR assay for stx1 and stx2.
Results: A total of 21 STEC isolates (39.6%) were shown to harbor the subA gene. SubA was detected is STEC isolates from all sources (cattle, sheep, human) with the exception of the pigeon's stx2f+ isolates. The presence of subA was more frequently associated with stx2+ as 66.6% (12/18) of isolates carrying this gene, and 53.8% (7/13) of stx1+/stx2+ isolates also harbored the Subtilase encoding gene. In contrast, only 20% of stx1+ isolates harbored this gene. Interestingly, one stx1+ isolate that were recovered from a diarrheic child was positive for this trait.
Conclusion: The results of the current study indicate the prominent distribution of Subtilase gene in STEC from different sources in Iran. The relevance of this trait to pathogenicity of the isolates should be addressed in future researches on clinical samples.

Keywords: Stec, Subtilase, Cattle, Human, Sheep, Iran

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SURVEY OF COEXISTENCE OF ESBLS WITH AMPC-B-LACTAMASES IN E.COLI IN Iran

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Background: The aim of this study was to determine the frequency of the coexistence of ESBLs with AmpC-β-lactamases in *E. coli* strains as a serious threat for treating bacterial infections.
Methods: During February to August 2013, 288 *E. coli* strains were isolated from clinical specimens in Rasht hospitals. After identification of isolates by biochemical standard methods, the antibiotic susceptibility test (Kirby-Bauer method) was performed according to CLSI standards against 20 antibiotics. The combined disk method (Double disk) was then carried out for detection of ESBLs producers *E.coli*.The blaCMY-2 gene was detected by PCR method using specific primers among ESBL positive strains. Then, PCR products were subjected to electrophoresis on 1.5 % agarose gel containing sybr safe. Finally, sequencing analysis confirmed PCR products as blaCMY-2 gene.
Results: A total of 288 clinical isolates, 118 (41%) were ESBL producers. The most prevalent ESBL producers were isolated from urine sample (102/118). *E. coli* strains showed the most resistance to Oxacillin (100%) followed by Ampicillin, Cephalotin and Amoxicillin (all of them 99.2%). Imipenem had the least resistance rate (0.8%) against all isolates. Among non β-lactam antibiotics, the highest resistance rate wasbelonging to Nalidixic acid, Tetracycline and Cotrimoxazole (66.7%, 64.6% and 63.9% respectively). Among 118 ESBL producers of *E. coli* only 7 cases (5.9%) were contained blaCMY-2 gene.
Conclusion: Our finding showed that the resistance rate to antibiotics was higher in ESBL producing *Klebsiella* spp. than non ESBL strains. 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 blaCMY-2. Therefore, one must look for other genes which confer ESBL production.

Keywords: *E.coli*, ESBL,Antibiotic Resistance, Blacmy-2

GENETIC BACKGROUND OF URINARY ESCHERICHIA COLI STRAINS AND FLUOROQUINOLONE RESISTANCE IN SELSELEH AND DELFAN REGION, LORESTAN, Iran

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Background: Phylogenetic background analyses have shown that *E. coli* strains fall into four main phylogenetic groups (A, B1, B2, and D) and that virulent extra-intestinal strains belong mainly to group B2; whereas most commensal strains belong to group A and to group B1. Previous studies have found that greater prevalence of biotype B2 was detected in susceptible than in fluoroquinolone-resistant *E. coli* strains. The aim of this study was to determine the distribution of phylogenetic groups and their association with fluoroquinolone susceptibility pattern among uropathogenic *E. coli* (UPEC) isolates in Selseleh and Delfan, Lorestan, Iran.
Methods: A total of 80 UPEC isolates were collected and biochemically identified from various hospitals in Selseleh and Delfan, Lorestan. After DNA extraction, phylogenetic groups were determined by using established multiplex PCR-based assays. Antibiotic susceptibility pattern of isolates was examined by disk diffusion method according to CLSI recommendations.
Results: The examined isolates (n=80) were distributed in phylogroups A (56.25 %), B1 (18.75 %), B2 (16.25 %) and D (8.75 %). Among 35(43.75%) ciprofloxacin (as a fluoroquinolone) susceptible isolates, the frequencies of groups A, B1, B2 and D were 62.85%, 22.85% and 14.28% and 0%, respectively; however among 45(56.25%) ciprofloxacin-resistant strains these frequencies were 22%, 17.77%, 17.77% and 15.55%, respectively.
Conclusion: The findings of our study showed that the phylogenetic group A was more prevalent among the tested isolates in our region. There are no significant association between the phylogenetic group B2 and susceptibility to fluoroquinolones. Although these results are consistent with those of other studies in Iran, they have considerable differences with some corresponding experiments in other parts of the world. It is also possible that the geographic source of isolates represents an important factor to be taken into consideration.

Keywords: Genetic Background, *Escherichia coli*, Fluoroquinolone

THE RELATIONSHIP BETWEEN VIRULENCE GENES AND O-SEROTYPE OF UROPATHOGENIC E. COLI IN ZABOL, Iran

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Background: *Escherichiacoli* strains that cause urinary tract infections possess several genes encoding urovirulent factors. They are classified based on various types of O antigen. For uropathogenic *E. coli*, the virulence factor profile of each strain is related with their O-serogroups. The aim of this study was to determine the relationship between eight virulence genes and twelve O-Serotypes of uropathogenic *E. coli.*
Methods: In this study, 100 *E. coli* samples were collected from patients with urinary tract infection from Zabol hospitals (Zabol, Iran). After the biochemical tests to confirm *E. coli* samples, DNA was extracted using boiling method. The identification of virulence factors and O-serogroups were performed by Multiplex PCR method.
Results: This study determined that fimH with 95% and cnf1 with 28% had the lowest and the highest presence rates of virulence genes, respectively. In addition, the presence of hlyA, iroN, iucD, iha, ompT and irp2 virulence genes were 32, 29, 69, 29, 67 and 89%, respectively.Of 100 *E. coli* samples, O types Prevalence include O1, O2, O4, O6, O7, O12, O15, O16, O18, O25, O75, and O157 were 6, 12, 6, 12, 2, 2, 5, 10, 3, 7 and 8, respectively. O15 type was found in none of the samples. fimH was associated with all the O-Serotypes.
Conclusion: This is the first report of *E. coli* serotyping in patients with urinary tract infection from the southeast of Iran and their relation to virulence genes.Based on these results, in most cases, there was no significant correlation between O- serotypes and virulence genes.

Keywords: O- Serotypes, Virulence Genes,Uropathogenic *E. coli*,Multiplex PCR, Urinary Tract Infection

PHYLOGENIC TYPING OF ESCHERICHIA COLI ISOLATED FROM BROILERS WITH COLLIBACILLOSIS IN TABRIZ, NORTH WEST OF Iran

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Background: In this study, to know about the phylogeny of *Escherichia coli* isolated from broilers with collibacillosis in Tabriz, 70 *E. coli* isolates recovered from broilers with collibacillosis were characterized for phylogenetic group (A, B1, B2, D) by multiplex PCR.
Methods: Bacterial isolates. Seventy *E. coli* isolates were recovered from broilers with collibacillosis in Tabriz, North West of Iran from different farms and confirmed according to the biochemical standards s described previously (Quinn et al 1994)and DNA extraction. Multiplex PCR for Phylogenic typing of *E. coli*. The phylogenic type was determined by multiplex PCR (Clermont et al 2000). Each 25 µl reaction contained 0.2 µl DNA templates, 1 U Taq DNA polymerase, 3.2 mM from primer (Table 1), 200 µM of each dNTP, 4 µl of 1 x PCR buffer, and 2 mM MgCl2.
Results: Of the all 70 samples, 35 (50%) isolates were classified as type A, 32 (45%) as type D, 2 (2.8%) as type B1 and 1 (2.8%) as type B2.
Conclusion: This study demonstrates the high prevalence of *E. coli* types A and D in infected broilers. This shows that the collibacilosis-causing *E. coli* bacteria are typical commensals, type A alongside pathogenic type, D in Iran. It is possible that this type of *E. coli* could acquire virulence genes from pathogenic types. Of course, such a claim needs further study.

Keywords: *Escherichiacoli*, Phylogeny, Collibacillosis, Tabriz, Broiler

DETECTION OF N-ACYL HOMOSERINE LACTONE (AHL) IN BIOFILM PRODUCING UROPATHOGENIC ESCHERICHIA COLI ISOLATED FROM URINARY TRACT INFECTION (UTI) SAMPLES.

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Background: N-Acyl homoserine (AHL) is the main component in quorum sensing and play an important role in biofilm formation. Little information is available regarding to AHL in *E.coli*. The purpose of this investigation was detection of AHL production among biofilm producing uropathogenic *E. coli* isolated from hospital in Kerman, Iran.
Methods: 36 uropathogenic *E.coli*strains were isolated from Afzal poor hospital during 6 months. The identity of bacterial species was confirmed by biochemical test. Biofilm analysis was done by microtiter plate at OD 490 nm. AHL was separated from cell mass supernatant by liquid-liquid extraction (LLE) and analyzed by colometric method. AHL functional groups were determined by Fourier transform infra- red spectroscopy (FT-IR).
Results: Among 123 urine sample collected from UTI patients 36 were confirmed as *E.coli*. The biofilm formation assay introduced 11 (30.55%) isolates that exhibited strong biofilm, 16 (44.44%) showed moderate and 9 (25%) demonstrated weak biofilm. Those uropathogenic *E. coli* with strong biofilm activity (9 isolates) were subjected to AHL detection. It was found that isolate no. 28 showed highest AHL activity (1.552) at OD520nm while, isolate no. 33 exhibited lowest amount of AHL (0.689). Two *E. coli* isolates that demonstrated the hight AHL were selected for FT-IR spectroscopy for analysis the functional group of AHL. A peak at 1764.33Cm-1 correspond to the C=O band of the lactone ring. The peak at 1377.99Cm-1 was related to N=H bond and lastly, a peak correspond to 1242.90 Cm-1 is for C-O bond.
Conclusion: From the above results it can be concluded that many *E. coli* isolates formed strong biofilm. This property along with AHL production may contribute in pathogenesis of the organism in UTI infection.

Keywords: Biofilm, Uropathogenic *E.coli*,AHL,UTI

COMPARISON BETWEEN THE COMMON FIMBARIE VIRULENCE FACTORS OF UROPATHOGENIC E.COLI BELONGING TO PHYLOGENETIC GROUP B2 AND A

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Background: Uropathogenic *E. coli* (UPEC) are the most important cause of urinary tract infections. Phylogenetic studies put *E. coli* into four main phylogenetic groups A, B1, B2 and D. The purpose of this study was to compare the Fimbarie virulence genes in B2 and A groups.
Methods: In this study, 100 *E. coli* samples were collected from patients with urinary tract infection in zahdan hospitals of Iran. After the biochemical tests to confirm *E. coli* samples, DNA was extracted by boiling method. The identification of virulence factors and phylogenetic groups were performed by Multiplex PCR method
Results: fimH, sfa and papC genes were present in 55(100%), 46(83%) and 46(83%) of samples belonging to Group B2, whereas in 14(82%), 12(70.58%) and 2(11%) of Group D samples, respectively.
Conclusion: This study determined that strains belonging to B2 group are the most important phylogenetic groups among *E. coli* strains causing urinary tract infection.

Keywords: Uropathogenic *E. coli* (UPEC), Urinary Tract Infections (UTI), Fimbriae, Virulence Factors

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DETERMINE THE PHYLOGENETIC GROUPS AND ACUITY IN FACTORS IN ESCHERICHIA COLI URINARY TRACT INFECTION

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Background: More than 80% of *E. coli* strains causing urinary tract infections in all age groups in the community. *E. coli* strains are divided into four phylogenetic groups.. Out of intestinal pathogenic isolates belonging to groups D and B2 are often the most acuity factors in compared to groups A and B1. The purpose of this study was to determine phylogenetic groups and the presence of acuity 8 factor (fimH, iucC,ibeA, sfa/foc, neuC, papC, afa, hly) in *Escherichia coli* was isolated from urine samples.
Methods: Over a quarter of positive cases of urinary tract infections caused by *Escherichia coli* had been collected. DNA extraction of the seprated isolates was done andthen PCR was performed using specific primers. The PCRswere electrophoresed to determine the groups of phylogeny and acuity in the factors. Analysis of the relationship between variables was done using SPSS software.
Results: From total of 60 *E. coli* bacteria, the highest frequency belong to group D (70%), Group A (3.23%) and B1 (7.6%), but none of the strains were belonging to group B2. Factors afa, sfa/foc,fimH,hly,neuC, andibeA were observed 53.3, 7.51%, 7.53%, 7.56%, 3.23%, 7.31%, 20 % and 3.73%, respectively. In this study, five acuity in factors papC,sfa/foc, fimH,ibeA,iucC showed a significant relationship with phylogenetic groups ($p < 0.05$).
Conclusion: The results showed that *Escherichia coli* urinary tract infection in the city of Jahrom were belonging to three phylogenetic groups A, B1 and D. 8 factor of acuity, fimH,iucC,ibeA,sfa/foc, neuC, papC, afa,hly were studied in this bacteria had varying frequency.

Keywords: *Escherichia coli,* Phylogenetic Group, Factors Acuity, Urinary Tract Infection

ANTIMICROBIAL RESISTANCE AND SURVEY OF PATHOGENIC GENES OF STX1 AND STX2 IN E.COLI O157: H7 ISOLATES FROM BEEF CATTLE AT INDUSTRIAL SLAUGHTER HOUSE OF FARS PROVINCE WITH MULTIPLEX PCR METHOD

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Background: *E. coli* O157: H7 is one of the most important pathogenic bacteria that produce, hemorrhagic colitis in humans. Livestock are the main reservoir of these bacteria. The aim of this study was to determine and evaluate the antibiotic resistance genes stx1 and stx2 in pathogenic *E. coli* O157: H7 isolates from beef cattle slaughter samples in the Fars province.
Methods: In order to study 360 samples of meat from cattle slaughtered in the spring and summer season of 1391, from slaughterhouses Kazeroon, Fassa and Shiraz was collected. Collected Samples was transferred to Kazaroun veterinary microbiology laboratory. Rapidly growing and microbial analysis of samples was performed and colonies suspected of *E. coli* O157: H7 were evaluated by PCR technique. After identification of bacterial, antibiotic-resistant strains were tested by disk diffusion method and specific primer of pathogenic-gene of 2 stx1 and stx2 with multiplex PCR method was evaluated.
Results: The results showed that from total 360 samples that collected in spring and summer Only 11 samples (05.3%), 3 in the spring and 9 in summer were contaminated with *E. coli* O157: H7.but pathogenic genes of Stx1 and stx2 are not found in any sample. Among the studied cities most polluted (6 examples) was associated with Kazeroon city. All bacteria were resisted against antibiotics such as penicillin, erythromycin and ampicillin. Also high percentage of them showed resistance to other antibiotics; 66.66 percent were resistant to cephalexin and Jntamysyn. But all isolated bacteria were sensitive to chloramphenicol.
Conclusion: The results showed a lower percentage of cattle beef at industrial slaughter province of Fars were infected by *E. coli* O157: H7. But a high percentage of isolated bacteria showed resistant to many antibiotics, especially gentamicin and cephalexin.

Keywords: *Escherichia Coli* O157: H7, Zoonoses, Antimicrobial Resistance, Pathogenic Genes

PHYLOTYPING OF ENTEROPATHOGENIC E. COLI ISOLATES FROM HOUSEHOLD DOGS WITH DIARRHEA IN KERMAN

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Background: *Escherichia coli* is presented as normal flora in the lower intestine of both humans and animals; however some strains can cause gastrointestinal illnesses. Most efforts have been put in the characterization of strains involved in intestinal disease in poultry, pigs, cattle and sheep, whereas lately *E. coli* that cause diarrhea in dogs have attracted more attention. The purpose of this study was to determine the phylogentic group/subgroup and the presence of the shiga toxin and intimin genes in *Escherichia coli* isolates from diarrheic dogs.
Methods: In this study, 63 fecal samples were collected from diarrheic dogs. Isolation and biochemical determination of *E. coli* strains were done. All the isolates were examined by PCR tests to determine the eae, stx1 and stx2 genes by multiplex PCR.
Results: According to the results, all of the isolates were negative for stx1 and stx2 genes. Four isolates (6/34%) were positive for eae gene. The eae positive isolates belonged to A (2 isolates), B2 (1 isolate) and D (1 isolate) phylogenic groups. These isolates belonged to A1 (2 isolates), B22 (1 isolate) and D1 (1 isolate) phylogenic subgroups.
Conclusion: Enteropathogenic *E. coli* pathotype have an important role in the pathogenesis of diarrhea in dogs.

Keywords: Phylogeny, Enteropathogenic, *E. coli*, Diarrhea, Dog, Iran

THE PREVALENCE OF ENTROPATHOGENIC AND SHIGA TOXIN PRODUCING E. COLI ISOLATES IN HEALTHY HOUSEHOLD CATS

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Background: *Escherichia coli* is a microbiota in intestinal tract of warm-blood animals. Pathogen strains can cause wide spectrum intra and extra intestinal diseases. Entropathogenic and shigatoxin producing *E. coli* strains are the most important causes of diarrhea in small animals. One of the most important virulence factors in EPECs is intimin (encoded by eae), while stx1 and stx2 toxins are very important in STECs. The aim of this study is to determine the entropathogenic and shiga toxin producing *E. coli* isolates among healthy household cats.
Methods: In this study total number 36 fecal samples were collected. After isolation *E. coli* strains were confirmed by biochemical tests and were subjected for detection of ipaH, eae, stx1, stx2 genes by multiplex PCR.
Results: Among the isolates 41/6% (n=15) had at least one of the eae, stx1, stx2 genes. The prevalence of the eae (66.6%) was more than the others. Also stx1, stx2 genes were found in 33.3% (n=5) isolates.
Conclusion: The results of this study show that in our country close contact with household cats plays an important role as a high risk factor in gastrointestinal infection.

Keywords: *Escherichia coli,* Cat, Entropathogenic And Shiga Toxin Producing

FREQUENCY OF FLUOROQUINOLONE RESISTANCE AMONG CLINICAL ISOLATES OF ESCHERICHIA COLI OF HOSPITALIZED PATIENTS IN INTENSIVE CARE UNITS OF QAZVIN, KARAJ AND TEHRAN TEACHING HOSPITALS

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Background: The aim of this study was to generate baseline data on the prevalence of fluoroquinolones resistance amongclinical isolates of *Escherichia coli* Hospitalized Patients in ICU of Qazvin, Karaj and Tehran teaching hospitals
Methods: In this study, from May 2012 to March 2013,210 *E. coli* isolates were collected from clinical sample of hospitalized patients in ICU. All isolates were initially screened for susceptibility to gatiflocxacine, levofloxacine, ofloxacine,norfloxacin,ciprofloxacin, nalidixic acid and ESBLs by disk diffusion method according to the CLSI 2013 guideline.
Results: Out of 210 isolates, 127(60.47%) isolates showed the reduction of susceptibility to fluoroquinolone and 111(87.40%) isolate were positive for ESBL production. Based on the study results, the resistance rates were as below: gatifloxacine 111(52.85%), levofloxacine 112(53/33%), ofloxacine 118 (56.19%), norfloxacin 115(54.28%), ciprofloxacin 121 (57.6%), nalidixic acid 127 (60.47%).
Conclusion: According to this study data, high rates of resistance to fluoroquinolone (as an important therapeutic agent recognized in clinical isolates of *E. coli* from hospitalized patients in ICU of Qazvin, Karaj and Tehran.

Keywords: *Escherichiacoli*, Fluoroquinolone Resistance, ICU

FREQUENCY OF ERMA,B,C GENES IN ERYTHROMYCIN RESISTANT ENTEROCOCCI ISOLATED FROM SAMPLES OF HOSPITALIZED PATIENTS IN UNIVERSITY HOSPITALS QAZVIN & TEHRAN CITIES

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Background: *Enterococci* are among the most important causes of healthcare associated infections, especially urinary tract infections with multiple drug resistance. The main mechanism of erythromycin resistance in *Enterococci* is 23SrRNA methylation, encoded by erythromycin ribosomal methylases (erm) genes. The aim of this study was to assess the frequency of erm A, B, and C genes in erythromycin-resistant *Enterococci* isolated from the clinical samples of inpatients of university teaching hospitals in Qazvin and Tehran.
Methods: From May 2012 to June 2013, a total of 165 samples of *Enterococci* were isolated from the clinical samples of inpatients of five teaching hospitals of Qazvin and Tehran. Suspected colonies on Azide Maltose Agar medium were identified by standard bacteriological methods. Susceptibility to erythromycin in *Enterococci* isolates was performed by agar dilution and disk diffusion methods according to the Clinical and Laboratory Standards Institute (CLSI) guideline. Erythromycin resistance genes (ermA, B, and C) were identified by PCR on erythromycin resistant and intermediate *Enterococci* isolates.
Results: Out of 165 clinical isolates of *enterococci*, 142 (86%) isolates were *Enterococcus faecalis* and 23 (13.9%) isolates *Enterococcus faecium*. The results of susceptibility test showed that 158 (% 95) isolates were resistant or with intermediate sensitivity to erythromycin. There was a good correlation between the results of disk diffusion and agar dilution methods. Of total samples, 147 (89%) isolates were resistant to erythromycin (MIC=8 µg/ml), 11 (6.6%) isolates with intermediate sensitivity (MIC 1-4 µg/ml), and 7 (4.2%) isolates with complete sensitivity to erythromycin (MIC= 0.5 µg/ml). MIC50 and MIC90 were 128µg/ml. A total of 147 (89%) isolates had MIC values equal to 128µg/ml. Frequency of methylase genes were: 41 (25.9%) for ermB; 64 (40.5%) for ermB and C; 14 (8.8%) for ermA and B; and 39 (24.6%) for ermA, B and C. Neither the ermA nor the ermC genes alone were reported for any of the study isolates.
Conclusion: The results of this study showed high prevalence of ermA, B, and C genes in erythromycin-resistant strains with ermB as the most common resistance gene among these isolates and detected in the all of intermediate or erythromycin resistant isolates.

Keywords: *Enterococci*, Erythromycin Resistance, Erma, B, C

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IDENTIFICATION AND ANTIMICROBIAL RESISTANCE OF HUMAN PATHOGENIC ENTEROCOCCUS SPP. ISOLATED FROM RIVER AND COASTAL WATER

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Background: The objectives of this study were to describe the species distribution and to evaluate the antimicrobial resistance of bacteria from the genus *Enterococcus*, which were isolated from river and coastal water.
Methods: The collected samples were identified to the genus level by Gram staining, catalase test, hydrolysis of bile-esculin and growth in NaCl 6.5% broth. Species identification was performed by PCR. One hundred and sixty-five (165) *Enterococci* isolates were tested for resistance to eight antibiotics. Diameters of zones of inhibition were recorded in millimetres and interpreted as sensitive or resistant.
Results: The *Enterococci* were detected in all of the analyzed water samples. In total, 70 *Enterococci* were isolated from Babolrud River and coastal waters of Babolsar, of these, 40 were isolated from Coastal water and 30 Babolrud river. Four different *Enterococcus* spp. were confirmed by PCR: *E. faecalis, E. faecium, E.gallinarum and E. casseliflavus*. The species distribution of isolates was as follows: *Enterococcus* faecalis (68.6%), *Enterococcus faecium* (20%), *Enterococcus gallinarum* (7.1%) and *Enterococcus casseliflavus* (4.3%) (Table1). The most frequently isolated *Enterococcus* species were *E. faecium* and *E. faecalis*. Resistance was highest to chloramphenicol (41.6) and least for vancomycin (8.3). *E. faeccium* showed the least resistance to vancomycin (7.1).
Conclusion: In the present study, we demonstrated the presence of *Enterococci* in all of the river and seawater samples analyzed. *E. faecalis* is the most frequent species isolated from River water, coastal bathing water samples (68.6%), and *E. faecium* is one of the major *Enterococcus* species in water samples (20%). Among the *Enterococcus* spp. isolated in current study 20% were resistant to ampicillin and 30 % of isolates exhibited resistance to ciprofloxacin.

Keywords: *Enterococcus* Spp., Antimicrobial Resistance, Coastal Water

DETECTION OF VANA GENOTYPE IN ENTEROCOCCUS GALLINARUM ISOLATED FROM POULTRY MEAT SAMPLES

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Background: This study was aimed to investigate the occurrence and molecular characterization of vancomycin susceptibility within *E. gallinarum* 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 assay. The isolates were confirmed as *E. gallinarum* by biochemical standard tests. They were further characterized by antibiotic susceptibility test. The agar dilution method was used for minimum inhibitory concentrations (MICs) determination. Finally, the strains were subjected to genetic analysis of vancomycin resistant gene, using specific primer sets.
Results: Totally 5 *E.gallinarum* were isolated including vancomycin-resistant *E. gallinarum* (VREG) (3), vancomycin-intermediate *E. gallinarum* (VIEG) (1), and vancomycin-susceptible *E. gallinarum* (VSEG) (1). The vancomycin MIC≥4 μg/ml was determined for testing vancomycin resistant gene except VSEG, four isolates were vanA carrier.
Conclusion: Up to our best knowledge, this is the first report of the occurrence of *E. gallinarum* carrying vanA gene in poultry meat. Our findings suggest that vanA gene could be taken up by *E. gallinarum* is as the transferable genetic element.

Keywords: *Enterococcus gallinarum*, Vancomycin Susceptibility, Vana, Poultry Meat, Iran

ISOLATION AND IDENTIFICATION OF ENTEROCOCCUS SPECIES FROM TRADITIONAL DAIRY PRODUCTS IN EAST-AZERBAIJAN, Iran

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Background: This study was conducted to determine the prevalence and diversity of *Enterococci* isolated from a variety of traditional dairy products.
Methods: A total of 90 samples consisting of cheese, yogurt, butter, cream, Kashk and butter milk were collected throughout East-Azarbaijan province. After preparation of sample suspensions/emulsions, they were cultured on Kanamycin Aesculin Azide agar plates. Typical colonies were subjected to standard morphological and biochemical tests such as Gram staining, catalase, growth in the presence of 6.5% salt, esculin hydrolysis, H2S production, motility, pigment production, acid production from L-arabinose, mannitol, lactose, D-raffinose, sucrose, argenine dihydrolysis, growth in the presence of 0.04% potassium tellurite and ability to growth in 4 °C.
Results: High loads of *Enterococci* contamination was found in most of the samples. Butter and cheese specimens demonstrated the highest contamination levels; meanwhile, the contamination level was the lowest in yogurt samples. Results revealed that butter had the highest *Enterococci* diversity compared to the other dairy products. Among the various species, *Enterococcus* faecium was estimated as the most frequent species. Furthermore, other species such as *E. fecalis, E. saccharolyticus, E. raffinosus, E. gallinarum and E. mundtii* were identified in different samples.
Conclusion: Fecal contamination of raw milk during hand-milking and/or environmental contaminations through non-hygienic practices during the manufacturing of traditional dairy products could be the major description for the high *Enterococci* loads in these products.

Keywords: Prevalence, *Enterococcus* Diversity, Dairy Products, East-Azerbaijan

PREVALENCE AND DIVERSITY OF ENTEROCOCCI IN RAW AND PASTEURIZED MILKS IN EAST-AZERBAIJAN, Iran

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Background: *Enterococci* have traditionally regarded as low grade pathogens emerged as an increasingly important cause of nosocomial infections. Due to their preferred intestinal habitat and their wide occurrence, *Enterococci* are used as indicators for fecal pollution assessing hygiene standards. The present study aimed to identify enterococcal isolates and species level from pasteurized and raw milks collected from retails and different bulk milk reception centers of East-Azerbaijan province, respectively.
Methods: *Enterococci* spp. were isolated and identified from different raw and pasteurized milks using standard morphological and biochemical tests such as Gram staining, catalase, growth in the presence of 6.5% salt, esculine hydrolysis, H2S production, motility, pigment production, acid production from L-arabinose, mannitol, lactose, D-raffinose, sucrose, argenine dihydrolysis, growth in the presence of 0.04% potassium tellurite and ability to growth in 4 °C.
Results: Occurrence of *Enterococci* was confirmed in 100% of raw and 37% of pasteurized milks. Upon our findings, 116 isolates were obtained from raw and 29 from pasteurized milk samples. The results depicted that *Enterococcus faecalis* and *E. faecium* were the prominent species isolates from raw and pasteurized milks, respectively. Furthermore, other types of *Enterococcus* spp. such as *E.gallinarum, E.saccharolyticus, E.sulfuresus, E.casseliflavus* and *E.faecalis* (avarient saccharolyticus) were isolated.
Conclusion: According to the results, routine speciation testing of *Enterococcus* in raw and pasteurized milks is emphasized due to the prevalence of wide variety of *Enterococci* species which useful indicator organisms for process hygiene and it has been noted that some *Enterococci* can survive pasteurization temperatures.

Keywords: Isolation, *Enterococci*, Raw Milk, Pasteurized Milk, Iran

ANTIMICROBIAL EFFECT OF CLOVE AND THYME OIL AND COMBINATIONS ON BIOFILM PRODUCING ENTEROCOCCUS FAECALIS

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Background: An important virulence factor of *Enterococcus faecalis*is its ability to form biofilm. The aim of this study was the activity of *Syzygium aromaticum* (clove) and Thymus vulgaris (thyme) and combinations together on *E.faecalis* biofilm formed in root canal.
Methods: Seventy human intact extracted single-rooted teeth with straight root canal randomly divided in to 5 groups: positive control (n=5), negative control (n=5), clove (n=20), thyme (n=20), clove and thyme (n=20). Each tooth was prepared with a crown down technique by using protaper rotary instruments. To formation of biofilm, samples were contaminated with *E.faecalis* and incubated at 37˚C in the sealed tube for 30 days. The SEM scan was performed to visualize the bacterial biofilm formation. The medicament correspond to the group was placed in the root canal. All specimens were incubated in 100% humidity and 37˚C for one week. Antibacterial activity was assayed against *E.faecalis* by using disc diffusion and microdilution method.
Results: Statistical analysis showed that clove and thyme alone cannot remove the totally biofilm however, their combined showed significantly lower efficacy compared to other groups
Conclusion: The cocentration 50 MIC of combination clove and thyme can to eliminate bacteria. Considering the non-toxic nature and other physiological benefits of this herbal oil therefore may be an acceptable instead Chemical irrigant.

Keywords: Biofilm, *Enterococcus faecalis*, Clove, Thyme

ISOLATION, IDENTIFICATION AND ANTIBIOTIC TYPING OF ENTEROCOCCUS SPECIES ISOLATED FROM COASTAL WATERS IN EASTERN REGIONS IN GUILAN PROVINCE

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Background: *Enterococci* are important human pathogens which involved either in community or hospital infections and exhibit intrinsic resistance to various antibiotics. In recent years, enterococcal infections have become major therapeutic challenge because of their increased incidence and spread of strains that have acquired resistance to several antimicrobial agents. Presence of bacteria in swimming waters is one of the contamination resources.
Methods: This study performed during suitable swimming seasons in summer and autumn. One hundred samples which collected in 14 prefectures in eastern regions of Guilan province were analyzed for the presence of resistant *Enterococci* to the common antibiotics. Also, we measured the parameters including temperature, turbidity, dissolved oxygen, pH, salinity, and phosphate and nitrate contents of waters. Enumeration of bacteria was achieved by MPN method. After confirmation of *Enterococci* species by biochemical and physiological tests, antibiotic susceptibility testing wasaccomplished by agar disk diffusion.
Results: The results showed that with decreasing of temperature, the number of *Enterococci* in different regions was reduced. The identified *Enterococci* species were included, *E. faecium, E. faecalis, E. casseliflavus,E. gallinarum, E. raffinosus, E. flavescens, E. hirae, E. solitaries,* and*E. durans*.
Conclusion: All of isolated *Enterococci* were resistant against Vancomycin, Penicillin, Ampicillin and Streptomycin. So, information about these bacteria could be useful in epidemiological and ecological studies.

Keywords: *Enterococci* Spp., Coastal Waters, MPN Method, Antibiotic Susceptibility Pattern

PREVALENCE OF GENES ENCODING AMINOGLYCOSIDE MODIFYING ENZYMES IN HIGH LEVEL AMINOGLYCOSIDE RESISTANT ENTEROCOCCUS CLINICAL ISOLATES BY PCR

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Background: The aim of this study was to determine the prevalence of high-level Gentamicin and Streptomycin resistant and mechanism of resistance to these antibiotics in the clinical isolates of *Enterococcus* spp.
Methods: From November 2011 to August 2013, a total of 195 enterococcal isolates were collected from clinical samples among 10 medical centers in Tehran, Iran. Confirmation of species was done by conventional tests (Arabinose, xylose, manitol, sorbitol and rhamnose fermentation). Anti-microbial susceptibility test was determined with disk diffusion according to CLSI (2013) guidelines. Detection of aminoglycoside resistance genes were done by PCR method.
Results: The isolates were found to consist of *E. faecalis* (65.12%), *E. faecium* (31.7%), *E. gallinarum* (2.56%) and *E. sulitarius* (0.51%).HLGR phenotype was detected in 35.51% of *E. faecalis* and 69.81% of *E. faecium* isolates. HLSR phenotype was detected in 40.18% of *E. faecalis* and 50.94% of *E. faecium* isolates. The results obtained from PCR showed a high prevalence of aac(6')-Ie-aph(2")-Ia gene among HLGR isolates. The aac (6')-Ie-aph (2")-Ia gene was identified in 87.77% of HLGR isolates. The ant (6')-Ia and ant (3″)-Ia genes were identified in 94.25% and 58.62% of HLSR isolates, respectively.
Conclusion: *E. faecalis* and *E. faecium* isolates differed in their susceptibilities to different antibiotics. Emergence of multi-resistant *Enterococci* and high level resistance to gentamicin and streptomycin showed by enterococcal strains is of concern because of the decrease in the therapeutic options for treatment of infections caused by *enterococci*.

Keywords: *Enterococcus* Spp, Antibiotics Resistance, HLAR, AME, Gentamicin, Streptomicin

DISTRIBUTION OF GELE,ESP AND HYL VIRULENCE GENES AMONG ENTEROCOCCUS FAECIUM ISOLATED FROM URBAN SEWAGE SAMPLES INTEHRAN

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Background: *Enterococci* (E) are facultative anaerobic Gram-positive cocci that tolerate extreme tempreture(10-45°C) and high sodium chloride concentrations. *E. fecalis* and *E. faecium* are common commensal organisms in the intestine of humans.They are causing severe urinary tract infections,wound infections, bacteremia, and bacterial endocarditis. The high level of antibiotic resistance(especially in *E. faecium*) and having several virulence factors increased pathogenicity of this genus.The presence of *E. faecium* in urban sewage correspond the transmission of infection diseases in persons.
Methods: In this study, 38 vancomycin-resistant *E.faecium* (VREfm) and 16 vancomycin-sensitive *E.faecium* (VRSfm) samples were isolated from urban sewage. All samples characterized by phenotyping (gram stain,catalase test,fermentation of lactose,6.5% NaCl and Bile esculin test) and MIC determination. Identification of virulence genes *gelE*,*esp* and *hyl* for each isolate was performed by PCR and specific primers at Microbiology laboratory of Faculty of Medicine of Shahid beheshti university of Tehran.
Results: PCR results showed the prevalence of virulence genes *gelE*(47.3%, 40%), *esp*(74.2%, 5.5%) and *hyl*(5.8%) found in VREfm and VSEfm, respectively.
Conclusion: The presence of multiple virulence genes in *E. faecium*isolated from sewage samples plays important role in the creation and development of diseases.Consequently, purification and chlorination of sewage are required for prevention or reduction of pollution by the microorganisms.

Keywords: *Enterococcus faecium*, Sewage,Virulence Genes

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EVALUATION OF GROWTH AND CELL MORPHOLOGY OF LISTERIA MONOCYTOGENES PTCC 1297 AS AFFECTED BY VARIOUS CONCENTRATIONS OF TOXIC HEAVY METALS

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Background: Heavy metal pollutants can localize and lay dormant, which can have severe effects on the environment. Unlike organic pollutants, heavy metals do not decay and thus pose a different kind of challenge for remediation. Microbial bioremediation is an efficient strategy due to its low cost and efficient alternative to target heavy metals. The objective of this study was to examine the viability and morphological characteristics of *Listeria monocytogenes* PTCC 1297 endured to toxic heavy metals.
Methods: Different concentrations of the heavy metals used in this study were rgBH2, PbO and CdSo4 (0.1%-0.5% wt/vol). All heavy metals were applied to exponential phase cells 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 and exposure of cells to selected concentrations of heavy metals, colony count of them was performed. Scanning electron microscopy (SEM) was implemented to visualize the surface appearance of bacteria after exposing to stress conditions.
Results: The HgBr2 at concentration of (0.1% wt/vol) and CdSo4 (0.2% wt/vol) were considered as lethal dose for *L. monocytogenes* PTCC 1297. Different concentrations of PbO could not kill *L. monocytogenes* but the rate of growth was decreased significantly. Additionally, morphologic and biochemical characteristics were changed significantly under each heavy metal concentrations.
Conclusion: The results indicated that *L. monocytogenes* PTCC 1297 had resistance after exposure to heavy metals. With more survey about tolerant bacteria to heavy metals there is possible to use them for bioremediation of water and waste water treatments.

Keywords: Heavy Metal Contamination, Bioremediation, *Listeria monocytogenes*

PREVALENCE AND FATE OF CLOSTRIDIUM DIFFICILE IN TWO TYPE OF WASTEWATER TREATMENT PLANTS

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Background: The frequency of community-associated *Clostridium difficile* infection (CDI) is increasingly recognized worldwide. Wastewater is a potential source for dissemination of *C. difficile* in the environment. The present study was conducted to determine the prevalence and fate of *C. difficile* in two types of wastewater treatment plants.
Methods: A total of 95 samples were taken from 5 different units of a conventional activated sludge system and 4 pound types of a waste stabilization pound system. Wastewater and sludge Samples from different parts of WWTPs were analyzed for total coliforms, fecal coliforms and *C. difficile*. Air and wastewater temperature were also measured.
Results: *C. difficile* was found in 13.6% (3/22) of digested sludge samples. However, no *C. difficile* was detected in inlet and outlet samples as well as raw sludge of conventional activated sludge system. *C. difficile* was also recovered from 5% (2/40) samples of waste stabilization pound treatment plant. The results of PCR assay showed that all isolated *C. difficile* were toxigenic strains (tcdB positive).
Conclusion: WWTPs are potential route for the dissemination of *C. difficile* in the environment and may act as a source of community-associated CDI.

Keywords: Waste Stabilization Pound, Conventional Activated Sludge, *C. difficile*, PCR

ISOLATION AND IDENTIFICATION OF CONTAMINANT MOLD OF THE IranIAN CARPET MAPS

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Background: This study aimed to survey the role of molds in biodeterioration of Iranian carpet map of national archives. In this study, 6 paper maps were randomly investigated.
Methods: Fungi were cultured onto Potato Dextrose Agar and Sabouraud Dextrose Agar.All isolates were subcultured on Potato Dextrose Agar. For microscopic observations, slide culture technique was carried out.
Results: In total, 24 fungal genera were isolated from the maps. Then, fungi were identified based on microscopic and macromorphological characteristics.
Conclusion: In this assay, *Aspergillus* sp., *Penicillium* sp., *Trichoderma* sp., *Chrysosporium* sp., *Ulocladium* sp. and *Cryptococcus* sp. were identified.

Keywords: Biodeterioration, Mold, Iranian Carpet Map

PRESENCE OF GIARDIA LAMBLIA IN POTABLE WATER OF CHAGARMAN PROVINCE, ANDIKA CITY

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Background: *Giardia* is a protoza that causes intestinal infection and diarrhea, especially in children of tropical zone. This type of protozoa is resistant to chlorine and can survive in the water for several days. *Giardia*sis causes impaired absorption of folic acid and vitamins, especially B12, which could leads anemia. Hence, the aim of this study is isolation and identification of *Giardia* from seven sample location of Andika city.
Methods: To evaluate the presence of *Giardia*, samples have been collected in 6 months from water of 7 stations in Andika city. The samples were transported to the microbiology lab and evaluated based on Iranian National Standard 5860. In addition, the patients with diarrhea who referred to health centers simultaneously were studied.
Results: The results obtained from this study showed that during 6 months sampling,only in July in a station in Chagarman, the organism was isolated, whereas, *Giardia* isolated and identified during same months from all patients referred to the health centers.
Conclusion: According to referred patients to health centers and presence of the organism in fecal and water samples in all studied months, the used system to remove the pathogenic organism in this area was not suitable.Furthermore, because of much presence of the organism in hot season and children who are an important group involved with *Giardia*, the local authorities must pay more attention to the new guidelines for removing the organism and the people who lives in this geographical area must train properly.

Keywords: Andika City, *Giardia*, Health Center, Water

ISOLATION AND IDENTIFICATION OF DIAZINON PESTICIDE DEGRADING BACTERIA FROM AGRICULTURAL SOIL MARVDASHT,FARS PROVINCE

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Background: The purpose of this study is isolation and identification of diazinon pesticide degrading bacteria and processing the growth kinetics in agricultural soils of Marvdasht, Fars province.
Methods: Sampling of three agricultural soils station polluted with diazinon in Marvdasht was done. Isolation of diazinon pesticide degrading bacteria in basic, salty Medium MSM was done. Assay of isolated bacteria in different concentration such as 0.2, 0.4, 0.6, 0.8 and 1 g/L diazinon was evaluated. For evaluation, the rates of the diazinon pesticide degrading used gas Chromatography.
Results: The most important of diazinon pesticide degrading bacteria were *Pseudomonas*, *Serratia*, *Flavobactrium*, and *Enterobacter*. All of the isolated bacteria showed high power of degrading.
Conclusion: In this study, the mean logarithmic number of bacteria in a medium containing diazinon 20*10-2 was lower than 10 * 10-4. The findings of this study showed that the best mixture of bacterial growth was in concentration 0.8 g/L with OD 1.327 in 84 hours.

Keywords: Biodegradation, *Serratia, Flavobacterium*

ISOLATION OF MODERATELY HALOPHILIC BACTERIA PRODUCING PULLULANASE ENZYME FROM DEGH BIARJEMAND DESERT OF SHAHROD, Iran

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Background: The main objective of this study was to isolate and identify moderately halophilic bacteria that produce saline-tolerant pullulanase enzyme in hypersaline condition.
Methods: The bacteria were isolated by saline culture from saline soils in Degh Biarjemand desert of Shahrod. Then moderately halophilic bacteria were isolated. On the next step, the isolated halophilic bacteria were analyzed for production of pullulanse enzyme. After that, they were identified by molecular and biochemical methods.
Results: A total of 11 strains were isolated from Biarjemand desert of Shahrod. 9 out of the 11 halophilic bacteria were moderately halophilic and the two others were halotolerant, but no extreme halphilic bacteria were among the isolated strains. The halophilic bacteria producing hydrolytic enzymes were analyzed. 2 strains of the bacteria produced pullulanase which one of them was moderately halophilic bacterium. The halophilic bacterium producing pullulanse enzyme was identified concerning the 16S rRNA sequencing technique as well as it's molecular and biochemical properties showed that the isolate belongs to the genus Bacillus.
Conclusion: In this study, for the first time, the halophilic bacteria from Degh Biarjemand desert of Shahrod were isolated and analyzed for production of pullulanase enzyme and special halophilic bacteria were isolated. The isolated halophilic bacterium produced saline-tolerant pullulanase enzyme, was tolerant and active in hypersaline condition.

Keywords: Halophilic Bacteria, Pullulanase Enzyme

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BACILLUSTHURINGIENSIS AND ITS EFFECTS ON GROWTH OF CAPSICUM ANNUUM

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Background: 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 and cultivation on nutrient agar and nutrient agar, the colonies with different morphology, color and 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 *Bacillus* 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 and ammonia production. The data were statistically analyzed using SPSS version 16.
Results: The pots, which inoculated with isolated Bacillus, 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 *Bacillus* and according to molecular identification the genus were*Bacillus thuringiensis* strain IAM 12077 (NR_043403.1). In addition, the isolates were able to produce indole acetic acid and this compound had effect on growth of capsicum annuum while the isolates were not able to produce ammonia.
Conclusion: *Bacillus thuringiensis* strain IAM 12077 (NR_043403.1) has a role on plant growth and there is possibility to use the isolates in further studies.

Keywords: *Bacillus thuringiensis*, Plant Growth, Capsicum Annuum

A COMPARATIVE STUDY OF BACILLUS SUBTILIS STRAINS ISOLATED FROM SOIL AND STANDARD STRAIN IN BIOLOGICAL CONTROL OF SOIL BORNE PLANT PATHOGENS

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Background: The aim of present study was to test the antifungal activity of *Bacillus subtilis* strains isolated from domestic soils against *Fusariummoniliforme* and *Verticilliumdahliae* compared with *Bacillus subtilis* ATCC21556.
Methods: *B. subtilis* strains were isolated from nine domestic soil samples. The isolates were screened by antifungal activity against *Fusarium moniliforme* and *Verticillium dahliae* and in order to the best production were optimized the culture conditions. The bacterial metabolites were obtained from 4 days grown isolates in optimized culture and iturin existent were confirmed by HPLC method. The iturin A (Sigma) and *B. subtilis* ATCC21556 were used as standards.
Results: 49 species from 180 colonies were isolated from soil samples and confirmed as *B. subtilis*. Three species were identified as the most antifungal active strains. Then nutrient broth with glucose and yeast extract as carbon and nitrogen sources, neutral pH and 30°C incubation temperature were optimized for best production. The HPLC chromatograms showed the extent of iturin A for three isolates *B. subtilis* like ATCC21556. One isolate identified as *Bacillus subtilis* 142 showed the best antifungal (iturin A) production.
Conclusion: Indigenous *B. subtilis* strain 142 produced lipopeptide antibiotic iturin A higher than standard strain and emphasized major role of iturin A in the antifungal activity of these *B.subtilis* against the target fungi. As a result we conclude *Bacillus subtilis* as biological control agents offer an alternative and supplement to synthetic pesticides.

Keywords: *Bacillus subtilis*, Biological Control, Fusarium Moniliforme, Iturin, Verticillium Dahliae

EVALUATION OF MICROBIAL CONTAMINATION AUTOMATED TELLER MACHINES (ATMS) IN HAMADAN CITY

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Background: Bacteria are ubiquitous organisms among the creatures that exist everywhere in the environment which able to grow on any surface. Continuous development and expansion of urbanization with increasing rate of population, and also limited times, cause people to use new developments in electronic banking which named Automated Teller Machines (ATMs). Today, the widespread use of electronic technologies as a source of health system contamination is considered. The aim of this study was to investigate the bacterial contamination on computer keyboards deployed at ATMs in Hamadan city.
Methods: The total of 360 ATMs at four locations in hamadan with the statistical calculations 96 ATMs were selected. Then, using sterile swabs soaked in normal saline, dragging on the keyboard surface sampling device ATMs was done. Swabs into the environment contain the nutrients such as nutrient broth was transferred to the microbiology laboratory of the University of Medical Sciences for being process.
Results: A Total of 65 samples of 96 different computer key boards available in ATMs of four areas were prepared. All of the samples 65 (68.42%) showed bacterial contamination. The collection of samples in the most infected computers' keyboards ATMs (Bank Melli18 samples (27.69%), saderat 12 samples (18.46%) were observed in Hamadan. 17 (26.15%) of bacterial pathogens had colonized the ATMs keyboard, while the 48 (73.84%) of them were contaminated with opportunistic organisms. The results showed that frequency of isolated bacteria included: *E. coli*, 6 (9.23%) for *Klebsiella* sp, 8 (12.3%) for *Entrobacter* sp. and 2 (3.07%) for (*Bacilluscereus* in 6 (9.23%), *BacillusSubtilis* 11 (16.92%) and family staphylococci *StaphylococcusEpidermidis* in 12 (18.46%), *Staphylococcusaureus* in (4.61%) and Micrococcaceae 5 (7.69%), *Pseudomonasaeruginosa* in 12 (18.46%) the prevalence of bacteria were isolated.
Conclusion: All keyboards tested had at least one species of bacterial contamination. Given these findings, it seems necessary to pay more attention to disinfect computer keyboards ATMs.

Keywords: Automated Teller Machines (Atms), Bacteria, Key Board

ISOLATION AND CHARACTERIZATION OF A NEW STRAIN OF ACHROMOBACTER SP. FROM ZEBRA SNAKE (SPALEROSOPHIS MICROLEPIS)

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Background: This is the first report of isolation of *Achromobacter* sp from Zebra snake (Spalerosophis microlepis).
Methods: Isolation was performed using an enrichment of current bacterial media with minor modification. The kidney was cultured in the appropriate medium and incubated overnight at 37ºC. After isolation DNA was extracted and PCR was carried out by 16S rRNA bacterial identification primer and PCR product was sequenced by BIONEER Company (Korea). Finally the sequences were compared with the GenBank database using the Basic Local Alignment Search Tool (BLAST) search.
Results: The unknown bacterium isolate was related to genus *Achromobacter*. They grow on current bacterial media in aerobic and anaerobic conditions after 48 h incubation at 30 ºC. They showed semi moisture round colony in culture. They are catalase-positive, oxidase-positive, gram-negative bacillus. According to gene bank and sequencing results, this is the first report of the isolation of *Achromobacter* from snake.
Conclusion: *Achromobacter* is a very uncommon cause of disease and found in host by accidental contamination. Although urinary system is not a common site for the subsequent development of *Achromobacter* but urine is the most common site from which *Achromobacter* is isolated in clinical microbiology laboratories. The capability of the *Achromobacter* to degrade naphthalene and other compounds completely and rapidly without the need to secrete biosurfactant may make it an ideal candidate to remediate PAH-contaminated sites.

Keywords: *Achromobacter*, Polycyclic Aromatic Hydrocarbons, Biodegradation

ISOLATION AND SCREENING OF INULINASE PRODUCING BACTERIAL STRAINS FROM SOIL

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Background: Microbial Inulinases constitute an important class of enzymes that degrade inulin into fructooligosaccharides and high-fructose syrup which are widely used in pharmaceutical and food industry. These enzymes are produced by various microorganisms including bacterial strains, filamentous fungi and yeasts. The aim of this study was to isolate and screen wild-type inulinase producing bacterial strains from soil samples of Gorgan district, Golestan province.
Methods: The soil samples were collected from different locations. The primary screening was performed based on hydrolytic zone on a inulin-based medium and Lugole`s iodine solution. Then morphological and biochemical characteristics of the isolated bacterial strains with inulinase activity were determined. Additionally, species-specific identification by 16S rDNA sequencing was performed on a few bacterial strains which had more inulinase activity.
Results: Nineteen inulinase producing bacterial strains were isolated from the soil samples. Out of Nineteen strains, 4 bacterial strains with more inulinase activity were identified by 16S rDNA sequencing. The species-specific identification revealed these 4 isolates as *Bacillu scereus* strain BF15, *Bacillus* sp.AK16, *Bacillus cereus* strain LD22 and *Enteroba ctercloacae* P101. *Bacillus pumilus* strain PIA39 was found the nearest homolog to the *Bacillus* sp.AK16.
Conclusion: We isolated and characterized inulinase producing bacterial strains from soil samples of Gorgan district. Most of them belonged to the *Bacillus* genus.

Keywords: Inulinase Producing Bacteria, Isolation, Soil, 16S Rdna Sequence

ISOLATION AND CHARACTERIZATION OF A CYANIDE DEGRADING BACILLUS SP. FROM CONTAMINATED SITE

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Background: The purpose of this study was isolation and characterization of cyanide degrading bacterium from contaminated areas.
Methods: After sampling of contaminated soil, cyanide degrading bacterium was isolated using enrichment culture method into the mineral salt medium M9 supplemented with cyanide. Degradation of cyanide was determined using picric acid method and increasing in bacterial growth using spectrophotometer.
Results: A rod shape, gram positive and spore forming bacterium with ability to degrade various combinations of cyanide was isolated then called *Bacillus* MS1. Also the isolate MS1 decomposed 2.46 mM cyanide within 38 hours.
Conclusion: These results indicated that *Bacillus* MS1 could be an appropriate option for cyanide degradation in industrial waste water and contaminated sites.

Keywords: *Bacillus*, Cyanide Degradation, Contaminated Sites

OPTIMIZATION OF ENVIRONMENTAL CONDITIONS OF AZO B-NAPHTOL ORANGE DYE DECOLORIZATION BY HALOPHILIC BACTERIA A3

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Background: The most common synthetic dyes which are used widely in textile, pharmaceutical and food industries are Azo dyes and because of Diazo links are considered as important biologically contaminants. Normally these dyes are resistant against conventional methods of wastewater treatment. Since nowadays, use of microorganisms for removal of azo dyes has been considered in literature.
Methods: Investigation on the extent of decolorization was carried out using variation of factors in each trial of experiment while other factors were constant as follows. The first step was preparation of base media of decolorization and pH adjusted to 6. Therefore, 15ml of decolorization media was added to test tubes and then various concentrations of Azo dyes were added and after 12days of incubation time at 35°C, the optimum concentration of 40 ppm was obtained. At the second step, concentration and temperature were constant at 40 ppm and 35°C and pH was varied from 6 to 10 and the optimum pH=6 was obtained. Similarly, at the final step, concentration and pH were constant at 40 ppm and 6 and temperature was varied from 35°C to 40°C and the optimum temperature was obtained 38°C. 5% (w/v) of NaCl in all decolorization media as regards of halophilic characteristics of bacteria is considered. The extent of decolorization was calculated according to the following formula using UV-Vis analyses before and after decolorization at λmax=480 nm. (%) D= (A0-A1)/A0×100 D = Extent of decolorization(%) A0 = Primary absorption (blank) at λmax=480 nm A = Culture media absorption after decolorization at λmax=480 nm.
Results: Optimum values for concentration, pH and temperature were determined as 40 ppm, 9 and 38°C, respectively. Under these optimal conditions, extent of decolorization after 12 days incubation time was obtained 60%.
Conclusion: We can conclude that the halophilic bacteria A3 can decolorize B-naphtol orange in environment.

Keywords: B-Naphtol Orange, Halophilic Bacteria A3, Decolorization

MEASUREMENT OF ETHANOL PRODUCTION BY ZYMOMONAS SPP. USING GREEN ALGAE

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Background: Due to rising world population, energy consumption in industrialized countries is increasing. For commercial production of biofuel,the using of fermentable organic waste such as algae is proposed. In this study, cheap carbon sources including green algae Spirogyra, Cladofora and Azolla were consumed for ethanol production by isolated *Zymomonas* spp.
Methods: Green algae were collected from shores of the Caspian Sea. Algae were dried using sun light then treated with dilute acids and bases. Liquid medium including 12% of the treated algae were prepared then Zymomonas ssp. were inoculated in aerated and non-aerated conditions at 30-35 °C.
Results: The results demonstrated that *Zymomonas* spp was produced about 14% ethanol using Cladofora that hydrolyzed with sodium hydroxide.
Conclusion: The result of this study revealed that xylose of green algae could introduce as a suitable and cheap carbon sources for bioethanol production.

Keywords: *Zymomonas*, Ethanol Production, Algae.

ISOLATION AND IDENTIFICATION OF ENTEROBACTER SP. RESISTANT TO POTASSIUM TELLURITE FROM QOM INDUSTRIAL WASTEWATER IN BIOREMEDIATION OF CONTAMINATED INDUSTRIAL ENVIRONMENTS

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Background: Abundant use of toxic oxianion of tellurite(TeO32-) in various industries such as textile, tannery, electroplating increase the environment pollution by toxic microorganisms and eukaryotes. Bioreduction of tellurite metalloid oxyanions in resistant bacteria in environments can be used as a valuable tool in biotechnology to detect the microorganisms for bioremediation of contaminated areas.
Methods: In this study,84 strains of bacteria were isolated from industry wastes. MIC (Minimum Inhibitory Concentration) determined with a concentration ranging from 0.1 to 26 mM potassium tellurite in 34°C, 7days and using agar dilution method. Then resistant strain was isolated that toleranced concentration of 23mM. In other researches,tolerate to concentration of 23mM was maximumt. The best strain was investigated by 16SrRNA and optimized in several ingredients such as different concentrations of tellurite, sodium chloride, pH, temperature and aerate.
Results: QWTmb2 strain isolated from waste textile according to 16SrRNA sequencing was recognized as *Enterobacter* sp.CCM6B.Using the spectrophotometric technique, the bacteriumeliminated 0.3mM of potassium tellurite in 12h. Maximum elimination in 24h observed in 250C,pH=7 and 50rpm.
Conclusion: This study presented QWTmb2 strain as an acceptable candidate for the elimination of tellurite in international societies.

Keywords: Oxianion, Tellurite, Bioremediation, Bioreduction

REMOVAL OF BACTERIAL AND FUNGAL CONTAMINATIONS OF WATER WITH ELECTRICITY

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Background: The purpose of this study was to evaluate the effect of electric current on bacteria *Escherichia coli, Staphylococcus aureus* and the yeast *Candida albicans*.
Methods: Bacteria and yeasts (lyophilized vials of Scientific and Industrial Research of Iran's central bank) were prepared. Suspensions of bacteria in distilled water were prepared. The count of bacteria in 1 ml of suspension was calculated as zero time. For electrolysis experiment, 100µl of microbial suspension was poured into the container. Electricity was stopped after 10 min, 15 min, 20 min, 25 min and 30 min of electrolysis. The different nutrient media were poured and after 18-24 hours of growth, the number of colonies was examined with three repetitions.
Results: The results showed that all bacteria and yeasts used in this study voltage 5/16 V are available annihilation. In this study, voltage 5/16 V and a current of 1 mA was identified and fixed that the number of bacteria (cfu/ml) *E. coli* at 25 and 30 min, *Staphylococcus aureus* at 10 min, and *Candida albicans* at 15 min significantly reduced.
Conclusion: The results showed that different species of microorganisms have different sensitivity to electrical current appears to increase the voltage or current at the time of placing greater inhibitory effect on the growth of microorganisms can be seen.

Keywords: Electric Current, *Escherichia coli, Staphylococcus aureus, Candida albicans*

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CHANGES IN DIVERSITY OF PSEUDOMONAS SPP. DURING BIOREMEDIATION OF POLYCYCLIC AROMATIC HYDROCARBONS

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Background: Here we studied the changes in diversity of *Pseudomonas* spp. during bioremediation of PAHs in soil microcosms prepared using soil samples gathered from KhangIran, Khorasan Province, Iran.
Methods: The polluted soil microcosm was constructed by addition of phenanthrene, antheracene, pyrene, and fluoranthene to the soil, each in 50 ppm concentration (M microcosm). Apparently non-polluted soil of the region was used as control (C microcosm). The microcosms were amended with nitrogen, phosphate and humidity and PAHs degradation were monitored in monthly intervals over a six months period. Each month total genomic DNA was extracted from soil using a Fast DNA SPIN Kit. *Pseudomonas* specific nested PCR combined with denaturing gradient gel electrophoresis (DGGE) were used to analyze the *Pseudomonas* community profiles. PCR product of excised and reamplified bands in DGGE's gel was sequenced and identified using Eztaxon.
Results: The result showed that *Pseudomonas cremoricolorata* and *Pseudomonas plecoglossicida* were dominant species in C microcosm and *Pseudomonas cremoricolorata* and *Pseudomonas plecoglossicida*, *Pseudomonas stutzeri* and *Pseudomonas toyotomiensis* were dominated in M microcosm. HPLC analysis showed that decreased 53.88%, 38.34%, 35.92%,52.38% phenantherne,antheracene,fluoranthene, and pyrene were degraded respectively in comparison to the first point.
Conclusion: *Pseudomonas stutzeri* and *Pseudomonas toyotomiensis* play important role in biodegradation of PAH.

Keywords: *Pseudomonas*,DGGE,HPLC,Eztaxon

EFFECT OF HEXADECANE ON BIOREMEDIATION RATE OF POLYCYCLIC AROMATIC HYDROCARBON

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Background: Here we studied the dynamics of the bacterial community, the number of heterotrophs and PAH degraders, during the bioremediation process of PAHs in presence and abcense of hexadecane.
Methods: Three microcosms prepared using soil sample gathered from KhangIran,khorasan Province Iran,The polluted soil microcosm was constructed by addition of phenantherene, antheracene, pyrene and fluoranthene to the soil, each in 50 ppm concentration with 1% hexadecane (MH microcosm) and the other one without hexadecane (M microcosm). Apparently non-polluted soil of the region was used as control (C microcosm). The microcosms were amended with additional nitrogen and phosphate sources. Humidity and PAHs degradation were monitored in monthly intervals over a six months period. PAHs and hexadecane degradation was monitored by HPLC and GC respectively and the bacterial number assessed by plate count method.
Results: At the first point the number of bacteria is almost the same in all of the three microcosms, but after 8 weeks the number of bacteria in the MH microcosm (1.85 × 107 per gram soil) was 15 times greater than M microcosm and 90 times greater than C microcosm. HPLC analysis showed increased PAHs degradation in MH microcosm, i.e. almost 1.5 times greater than M microcosm. During the bioremediation process more than 70% of initial concentration of phenanthrene and Pyrene were disappeared.
Conclusion: The findings revealed that PAHs degradation is significantly increased in the presence of hexadecane likely due to increasing the bioavailabilty of PAHs and number of oil degraders by hexadecane.

Keywords: Microcosm,Hexadecane,Plate Count,HPLC

ACTINOMYCETES WITH AMYLOLYTIC ACTIVITY ISOLATED FROM SEDIMENTS OF THE CASPIAN SEA

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Background: The aims of this study were isolation and characterization of actinomycetes with amylolyticactivity from sediment of the Caspian Sea.
Methods: For isolation of actinomycetes, sediments were collected in different depth of water. Plates were incubated for 21 days at 28 °C after inoculation of samples into Starch Casein Agar (SCA) as a selective medium. Colonies with a tough or powdery texture were isolated.Primary screening was performed by the starch plate assay method and amylolytic activity of isolates was confirmed by formation of clear zones of hydrolysis around the colonies. It was also determined by DNS (3, 5-dinitrosalicylic acid) assay method as secondary screening.
Results: Of the sediments, 14actinomycetes were isolated and identified.All of them were determinedas active actinomycetes in primary screening. Among thesebacteria, five isolatesshowed good ability to produce amylasein secondary screening.
Conclusion: The result of this study demonstrated that the sediments of the Caspian Sea have the potential to be a diversity of actinomycetes with amylolytic activity.

Keywords: Marine Actinomycetes, Caspian Sea, Amylolytic Enzymes

ISOLATION AND IDENTIFICATION OF CADMIUM-RESISTANT BACTERIA FROM WASTEWATER TREATMENT PLANT OF SHAHINSHAHR IN ISFAHAN

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Background: Heavy metals enter the environment through industrial activities and among the most toxic heavy metal in the environment are the Cadmium.This metal can eventually reach the tissues of humans and animals. In this study, the Cadmium-resistant bacteria from effluent of wastewater treatment plant of Shahinshar were investigated.
Methods: Sampling of effluent of the wastewater treatment plant of Shahinshahr was taken and the level of Cadmium samples was measured by the atomic absorption device. For the removal of cadmium-resistant bacteria, serial dilutions of the samples were prepared and the dilutions were cultured on the PHG II culture medium, containing density 0.5 mM cadmium nitrate.Then, bacterial identification was done by morphological and biochemical tests and their molecular identification are underway.
Results: In this study, the level of cadmium presents in wastewater samples was equals to 0.005 ppm. Cadmium-resistant isolates were detected based on biochemical tests of *Pseudomonas aeruginosa* strains, *Micrococcus luteus* and *Micrococcus roseus*.
Conclusion: The findings of this study can be concluded that the removal of heavy metals from wastewater by metal-resistant bacteria can fix solution to environmental problems created by the industry, as well as a good alternative to conventional chemical and physical methods for removal of toxic metals.

Keywords: Cadmium, Wastewater, Metalresistant Bacteria

ISOLATION AND MOLECULAR IDENTIFICATION OF L-ASPARAGINASE PRODUCING BACTERIA FROM PERSIAN GULF

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Background: L-asparaginase is an anti-neoplastic agent used in the chemotherapy of lymphoblastic leukemia. Nowadays, this enzyme is derived from bacterial sources. The objective of this study was isolation and molecular identification of L-asparaginase producing bacteria from marine environment in Persian Gulf.
Methods: 53 marine bacteria were isolated from sediment and seawater samples obtained from Persian Gulf in Hormozgan, Iran. The isolated strains were screened for L-asparaginase activity. Production of L-asparaginase was carried out in medium M9. Based on the enzyme activity among 20 strains producing L-asparaginase, 4 isolates with high L-asparaginase activity were selected and identified base on nucleotide sequence of 16S rRNA gene.
Results: 4 bacteria isolated were identified as *Pseudomonas* strains and four strains of *Bacillus* sp. and*Pseudomonas* sp.(PG01) showed the highest productivity of 1.6 IU, and *Bacillus* sp. (PG04) showed least productivity of 0.67 IU.
Conclusion: This study revealed that bacteria isolated from Persian Gulf may be potential source of enzyme L-asparaginase and*Pseudomonas* sp.(PG01) with high productivity L-asparaginase can used for chemotherapy of acute lymphoblastic leukemia, biosynthesis of the aspartic family of amino acids and production decrease acrylamide in food industrial.

Keywords: L-Asparaginase, Marine Bacteria, 16S Rrna, Persian Gulf

ISOLATION AND IDENTIFICATION OF RAIN-MAKING BACTERIA

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Background: Ice nucleation active (INA) bacteria have play a role in atmospheric processes and participate in assort of biological precipitation cycle. The most surveys on these bacteria were from subtropical area, so, studies of INA bacteria from other area are needed. The aim of current research was isolation and identification of INA bacteria from north of Tehran, Iran to investigate their ice nucleation property.
Methods: Bacterial genus were isolated from plant surface (Japan allspice) and winter precipitation (snow and rain) after culturing on BHI medium and incubated in a various temperatures (4, 10, 15, 20 and 25°C) for 24-96 hours. Droplet freezing method was used to screen potential of ice nucleation producing bacteria. 16S rRNA analysis was applied to confirm genus and species of the isolates. Outer membrane protein of ice nucleation positive strain was purified and identified by SDS-PAGE method.
Results: The results showed that among 50 isolates, 40 strains had the ability to produce ice nucleation and 10 strains showed extremely high ice nucleation activity. Gram positive cocobacilli (5%), gram negative bacilli and gram positive cocci (25%) were determined as the least and the most microbial populations, respectively.
Conclusion: The best strain was a gram-negative rod shape with the ability of complete freezing of water in less than 5 minutes. It was identified as *Pseudomonas syringea* based on 16S rRNA gene sequencing. Selected isolate in this research could be a promising tool for biopercipitation.

Keywords: Bioprecipitation, *Pseudomonas syringe*, Droplet Freezing Method, INA Bacteria

BIODEGRADATION OF PHENOL BY THE YEAST TRICHOSPORON CUTANEUM

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Background: The aim of this study was to biodegrade phenol by the yeast *Trichosporon cutaneum*.
Methods: In this study, the phenol-degrading yeast from activated sludge was isolated from Sewage refinery in Isfahan. After three passages, two yeasts isolated. One Yeast that had higher growth rate was selected for more study. Also, this yeast was able to remove phenol that was measured by Gibbs reagent. The effect of four different concentrations of phenol (50, 125, 200 and 275 mgl-1) was measured. The hydrophobicity and emulsification activity was measured in yeasts. Finally,the yeast was identified by molecular method by amplification of 18S rRNA gene region.
Results: The yeast had the high growth rate in the presence of phenol as only energy and carbon source. Also this strain had high percentage of phenol degradation. The strain had the maximum growth in 0.05 gl-1 concentrations. Molecular identification shows that the strain is related to *Trichosporon cutaneum*.
Conclusion: *Trichosporon cutaneum* had high percentage of phenol degradation and we can use this yeast for removing phenol of industrial wastewaters.

Keywords: Biodegradation, Phenol, Yeast

THE PREVALENCE OF ESCHERICHIA COLI STRAINS HARBORING IPAH VIRULENCE GENE ISOLATED FROM SURFACE WATER SOURCES OF TEHRAN PROVINCE.

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Background: *E. coli* is considered as one of the most important causes of bacterial infections transmitted through water and foods. Several factors are involved in the pathogenesis of *E. coli* including *ipaH* gene related to entroinvasive pathotype. The aim of this study was to investigate the prevalence of *E. coli* strains carrying virulence gene *ipaH* isolated from different surface water sources in Alborz province.
Methods: This study was carried out in 2013. The research included all *E. coli* strains isolated from different surface water sources of Tehran province including input of 5 water treatment centers. *E. coli* isolates were detected and identified by standard microbiological and biochemical tests. The strains were evaluated for presence of virulence gene *ipaH* by PCR using specific primers. The PCR amplicons were visualized after electrophoresis and staining with SYBR green.
Results: One hundred and six *E. coli* strains were isolated and included in the study. The PCR results showed 4 strains harbored *ipaH* gene. Approximately, we found an equal rate of ETEC detection among the strains isolated from different water source. Most of bacterial strains harboring *ipaH* gene were MDR.
Conclusion: Our finding showed the prevalence of virulence gene *ipaH* is not so much high among *E. coli* strains isolated from different surface water sources in Tehran province. Considering its plasmid borne nature, the risk of transmission of this gene between other bacterial species could pose a high threat for public health.

Keywords: *E. coli*, Ipah, PCR, Water.

THE PREVALENCE OF ESCHERICHIACOLI STRAINS CARRYING VIRULENCE GENE ESTA AND ELT ISOLATED FROM DIFFERENT SURFACE WATER SOURCES OF TEHRAN PROVINCE

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Background: The aim of this study was to investigate the prevalence of *E. coli* strains carrying virulence gene *estA* and *elt* isolated from different water sources of Tehran province.
Methods: This study was carried out in 2013. The research included all *E. coli* strains isolated from different surface water sources of Tehran province including input of 5 water treatment centers. *E. coli* isolates were detected and identified by standard microbiological and biochemical tests. The strains were evaluated for presence of virulence genes *estA* and *elt* by PCR using specific primers. The PCR amplicons were visualized after electrophoresis and staining with SYBR green.
Results: One hundred and six strains were isolated and included in the study. The PCR results showed 10 strains harboring both *estA* and *elt* genes. Approximately, we found an equal rate of ETEC among the strains isolated from different water source. Most of bacterial strains belonging to ETEC pathotype were MDR.
Conclusion: Our finding showed the frequent prevalence rate of virulence gene ETEC pathotype among strains isolated from different surface water sources in Tehran province. Considering its plasmid borne nature, the risk of transmission of this gene between other bacterial species could pose a high threat for public health.

Keywords: *E. coli*, *Esta*, PCR, Water

ISOLATION AND IDENTIFICATION OF PHENANTHRENE AND PYRENE-DEGRADING BACTERIA FROM PAH-CONTAMINATED SOILS

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Background: In this study we isolated and identified bacterial strains with the ability of degrading the two polycyclic aromatic hydrocarbons (PAH), phenanthrene and pyrene.
Methods: The PHA degrading bacteria were isolated from PAH-contaminated soils around Tabriz and identified using 16S rRNA sequencing. The isolates had optimum growth at 32 ˚C and pH 7.2 on mineral salts medium (MSM) with 400mg/l phenanthrene or pyrene as the sole carbon source. The isolates Phenanthrene degradation efficacies of the isolates were determined in the broth medium and remaining phenanthrene concentration was measured using a gas-chromatograph.
Results: The bacterial strains was determined as *Pseudomonas* sp.The GC results showed that about 98 percent of the PAH had been degraded.
Conclusion: Identification of the bacterial strains with the ability of degrading polycyclic aromatic hydrocarbons(PAH) can be used to remove them and to protect human and environmental health.

Keywords: Degrading Bacteria, Phenenthrene, Pyrene, Polycyclic Aromatic Hydrocarbons

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ENRICHMENT, ISOLATION AND IDENTIFICATION OF MTBE- DEGRADING BACTERIAL FROM WASTEWATER MTBE PLANT IN MAHSHAHR

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Background: The aim of this work was to isolate and identify soil microorganism capable of degrading MTBE.
Methods: Samples collected from a wastewater MTBE plant of Mahshahr Petrochemical, Iran, and carried in screw-cap bottle to laboratory. Enrichment technique was used to isolate MTBE degrader. In enrichment studies, a Basal Salts Medium (BSM) was utilized which supplemented with MTBE compound in 6 concentration 50-800 ppm as sole source of carbon and energy. Contaminated soils also used as inoculums in enrichment steps.
Results: According to this research, the consortium obtained from the wastewater of MTBE plant was performed by periodically adding MTBE over 3-month period. After several enrichment steps in BSM liquid medium containing MTBE as the sole carbon, of 800ppm Concentration MTBE, a green- blue pigmented strain pw3, (i.e. *Pseudomonas aeruginosa*) which showed faster rate of MTBE removal, was isolated. It is worth nothing that this bacterium was capable to grow in MTBE concentration of 600ppm as sole carbon and energy source. Pure culture was identified by colony morphology, Gram stain reaction and biochemical tests. Molecular identification study (PCR) to be continued
Conclusion: It is well known that MTBE-degrading bacteria play an important role in the elimination of MTBE in soil. Biodegradation is one of the promising techniques to reduce MTBE contaminated in the environment and MTBE degraders were reported as an efficient method used to degrade MTBE. The results of our research showed the potential pure bacterial cultures of *Pseudomonas* sp. For biodegradation of the MTBE-contaminated sites and confirmed the significant ecological role of bacteria in petroleum-polluted environments.

Keywords: Biodegradation, Mtbe

ISOLATION OF MTBE-DEGRADING BACTERIAL FROM GASOLINE CONTAMINATED SOIL IN KHUZESTAN

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Background: The aim of this work was to isolate and identify soil microorganism cable of degrading MTBE.
Methods: Sampling Soil samples were taken from the depth of 5-30 cm of gasoline contaminated soils in Khuzestan, Iran, and carried in screw-cap bottle to laboratory. Soil samples were passed through 2mm sieve to prepare them for further microbiological examination. Enrichment culture Enrichment technique was used to isolate MTBE degrader. In enrichment studies, a Basal Salts Medium (BSM) was utilized which supplemented with MTBE compound in 6 concentration 50-600 ppm as sole source of carbon and energy. Contaminated soils also used as inoculums in enrichment steps
Results: According to this research of 600 ppm concentration MTBE, a gram positive aerobic bacteria was isolated, (i.e. *Bacillus coagulans*), it is worth nothing that this bacteria was capable to grow in MTBE concentration of 600ppm as sole carbon and energy source. Pure culture was identified by colony morphology, Gram stain reaction and biochemical tests. Molecular identification study (PCR) to be continued.
Conclusion: It is well known that MTBE-degrading bacteria play an important role in the elimination of MTBE in soil. Degradation is enhanced at some sites because of chemical-induced selection or adaptation of microorganisms resulting from chronic exposure to chemical. Isolation, determination, and characterization of microorganisms which participate in biodegradation of methyl tertiary-butyl ether (MTBE) have great significance in the decontamination of environment in shorter periods.

Keywords: Pure Culture, Contaminate Soil, Mtbe

EFFECTS OF MEDIA NUTRIENTS AND CULTIVATION CONDITIONS ON BIOMASS PRODUCTION OF FRESHWATER MICROALGA CHLORELLA VULGARIS

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Background: Chlorella vulgaris is a freshwater microalga capable of accumulating high lipid content, possessing high growth rate, providing suitable fatty acid profile for biodiesel production and also it is widely cultivated with inexpensive nutrient regime.
Methods: In this study, the growth rate and biomass production of C. vulgaris were investigated under different culture media namely Bold Basal medium (BBM), Modified Bristol's medium (MBM), Blue green algae-11 medium (BG-11), Rudic medium and Zarrouk medium.
Results: Culture of C. vulgaris in Zarrouk medium caused higher growth rate and higher biomass production. In addition, different cultivation modes, phototrophic (NaHCO3), photoheterotrophic (molasses) and mixotrophic (NaHCO3 and molasses) modes were examined to determine the efficient culture condition. Our results revealed that the highest biomass productivity of C. vulgaris could be obtained in cultures under mixotrophic conditions. This study also highlights the possibility of using waste molasses as a cheap carbon source and as alternative to glucose-based medium in C. vulgaris culture.
Conclusion: A higher concentration of NaHCO3 exhibited longer lag phase and slow growth rates because salinity generated by Na+ can inhibit the growth of the freshwater microalgae, C. vulgaris.

Keywords: Chlorella Vulgaris, Medium Composition, Cultivation Modes, Waste Molasses

ISOLATION AND CHARACTERIZATION OF METHANE-OXIDIZING BACTERIA FROM SOIL

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Background: The main purpose of current study is isolation and characterization of the bacteria which are capable of methane consumptionas the sole source carbon and energy.
Methods: Soil samples were collected from the oil contaminated regions. The bacterial suspension was prepared and inoculated into NMS (Nitrate mineral salts) broth medium. Then atmosphere of the flasks werefilled up with 50% of methane and 50% of air.Incubation was performed on the shaker for 10 days in30°C in the dark condition. In addition, the gas mixture was renewed every 2 days. Then liquid culture samples werespread onto NMS agar plates. The plates were incubated for 2 weeks in a gas-tight jar with similar gas composition as mentioned above. Single colonies thatformed on the NMS agar plates were transferred onto fresh NMS agar plates and then incubatedfor another week. The contamination of samples with non-methane oxidizing bacteria was investigated by incubation of control plates in the absence of methane.Identificationof bacteria was carried out using biochemical tests and molecular procedure.
Results: In this research, 2 bacterial strains were isolated from the oil contaminated soils. On the basis of morphological, physiological properties and biochemical characteristics such as phenylalanine deaminase, DNase, hydrolysis of esculin, tween 80, starch, gelatin, and 16S rRNA gene sequencing analysis,the organisms were identified to belong to Achromobacter and Sphingomonas genera.
Conclusion: Microbial prospection for oil and gas (MPOG), is a surface exploration technology based on the detection of anomalies in microbial distribution in soil samples. Geomicrobial prospecting for hydrocarbons is an exploration method based on the seepage of light gaseous hydrocarbons from oil/gas reservoirs towards the surface and their utilization by hydrocarbon-oxidizing bacteria.

Keywords: Microbial Prospection, Methane Oxidizing Bacteria, Oil, Gas

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ISOLATION AND CHARACTERIZATION OF ANTIBIOTIC PRODUCER ACTINOMYCETES SPECIES FROM THE PERSIAN GULF

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Background: To evaluate the antimicrobial activity of actinomycetes isolated from the Persian Gulf, as untapped source for searching new natural antibiotics.
Methods: Initially, water and sediment samples were collected from 23 study sites in the some northwest region of Persian Gulf. Isolation was done using Starch casein agar and ISP2 agar (Yeast extract - malt extract agar). Inhibition effects of their extracellular broth were assessed against 5 Gram positive and 4 Gram negative bacteria by top agar layer method. Also pure colonies of bacteria isolates were inoculated into ISP2 broth medium and were incubated on a rotatory shaker (200 rpm) at 28°C for 7-10 days to produce secondary metabolites. Then supernatant was extracted using the solvents such as ethyl acetate, methanol, chloroform, acetone and etc. The optimized solvent was ethyl acetate. Bioactivity of their ethyl acetate extract was assessed at 100 mg/ml concentration by disk diffusion method. Synthetic antibiotics were used as control. Characterization of the best producers was carried out by morphological, physiological, chemotaxonomic and biochemical methods.
Results: 35 strains of actinomycetes were isolated from samples. 18 isolates showed antibacterial activity against at least one of the bacteria tested with more than 15mm diameter of inhibition zone and 5 isolated had more than 25mm diameter of inhibition zone. SSC5 and BW5 strains inhibited grow of all of the bacteria tested and had more than 30mm diameter of inhibition zone, therefor selected for further investigations. The crude extract had higher inhibition zone against Gram negative bacteria than Gram positive bacteria. SSC5 and BW5 strains were identified as *Streptomyces* sp. using shirling and Gottlieb 1966 with bergeys manual of determinative bacteriology.
Conclusion: Multidrug resistance organisms play a dominant role in many clinical problems globally. Therefore, there is an urgent need for developing new drugs which are effective against current antibiotic resistant pathogens. Natural compounds obtain from marine source play important key to discover various new drug. Actinomycetes are the most potent industrially important the microorganisms which are capable for the produce bioactive compounds like antibiotic.

Keywords: Persian Gulf, Actinomycetes, Disk Diffusion Method, Antibiotic

ELECTRICITY GENERATION BY ESCHERICHIA COLI FROM MOLASSES IN A MICROBIAL FUEL CELL

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Background: Microbial fuel cells (MFC) are new technology devices for electricity generation from microbial oxidation of organic substrates. MFC is environmentally-friendly process produces electricity by using bacteria to convert organic waste material into electrical energy. The aim of this investigation was to assess the ability of *Escherichia coli* to produce electricity from molasses in microbial fuel cell system.
Methods: In this study dual chamber microbial fuel cells were constructed with glass and using graphite plates as electrodes. The volume of each chamber being 160ml was separated by a Flemion membrane. Pure culture of *Escherichia coli* cells was inoculated into MFC. Methyl red was employed as mediator for electron transfer from *E. coli* to graphite anode electrode. Molasses with a concentration of 3% was used as feed in the anodic chamber. The performance of MFC was evaluated by voltage-power polarization measurement.
Results: Maximum power density was 143.44 μw/m2 at a resistance of 2.2 KΩ and maximum current density was 2.11 mA/m2. After 80 hours of MFC operation the voltage of cell dropped. So, 140 ml from anode chamber was replaced with fresh medium, which causes the voltage increase again.
Conclusion: The results showed the molasses could be a suitable substrate to produce electricity in MFC system by *E. coli*.

Keywords: Microbial Fuel Cell, Molasses, *Escherichia coli*

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HYPERTHERMOPHILIC NANOBACTERIA ISOLATED FROM GHEINARCHEH HOT SPRING

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Background: The aim of this research was to isolate and identify the thermophilic nanobacteria from Gheinarcheh hot spring.
Methods: Microbial mats were found from Gheinarcheh hot spring in the north west of Iran (Latitude: 38° 16' 1.3012", Longitude: E 47° 48' 35.1123") and we tried to isolated protease producing thermophilic microorganisms. To identify features of these mats, synthetic media was used and incubated in temperature range between 60 to 1200 °C also in autoclave. To describe this microbial biofilms, scanning electron microscopy was applied and DNA extraction was done with method of Sheima and PCR was performed with four universal 16S rRNA primers.
Results: Microbial biofilms were discovered to be present in developmental stages of biofilm formation observed in our samples and was increased in high temperature between 60 until 1200 °C and also in autoclave quickly. In triplicate SEM samples has been seen as the nano-sized features in the scale of 70 nm as nanobacteria. DNA extracted was seen with gel electrophoresis and PCR was not performed with the universal 16S rRNA primers.
Conclusion: The present work involves the isolation and characterization of thermostable nanobacteria. Superficial and development recognitions of these thermophilic nanobacteria were identified from Gheinarcheh hot spring.

Keywords: Nanobacteria, Hot Spring, Hyper-Thermophilic

INVESTIGATION OF THE ELECTROCOAGULATION IN HARVESTING CHLORELLA VULGARIS FOR BIODIESEL PRODUCTION

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Background: One of the most critical steps in production process of biodiesel using microalgae biomass is harvesting of microalgae that contributes about 20-30% of the total biomass production cost. The aim of this study is to optimize microalgae biomass harvesting by electrocoagulation.
Methods: In this experiment, Chlorella vulgaris was cultivated in municipal wastewater with preliminary treatment, under 3000 lux illumination, 30° C and 130 rpm rotational speed. The experiments were performed after five days of cultivation, at the end of logarithmic phase. One and two couples of Aluminum electrodes were used and the culture volume used for each experiment was 250 ml. Parameters examined in this study were current density (7.9 and 80 A/m2), the distance between electrodes (1 and 2 cm), pH (5 and 10), agitation speed (0 and 100 rpm) and time (2 and 15 min). The effects of parameters were evaluated by fractional factorial using design expert software. The separation efficiency is determined by optical density changes.
Results: According to the results, alkaline pH is suitable. By increasing agitation speed, the foam was produced in the culture; result in dispersion of flocculations and decreasing separation efficiency. Another result of this study was that increasing the number of electrodes and time cause higher efficiency but the gradient of the efficiency versus time is much lower. Current density and distances between electrodes interact.
Conclusion: The results show that as compared to other harvesting methods; electrocoagulation is faster, easier and more economical technique for scale up at industrial scale.

Keywords: Electrocoagulation, Chlorella Vulgaris, Biodiesel

MOLECULAR DETECTION OF CAMPYLOBACTER SPP. ISOLATED FROM CASPIAN SEA'S WATER SAMPLES IN NORTH OF Iran

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Background: *Campylobacter* is one of the main causes of gastroenteritis in humans. This bacterium can enter into the human body through the consumption of contaminated water and animal food products. The major purpose of this study was isolation and identification of *Campylobacter* spp. from Caspian Sea's water in north of Iran.
Methods: *Campylobacter* spp. was isolated using pret-KB method and then identified by phenotyping tests, which are introduced by Attabay and Corry. Finally, the identification of strain was verified by PCR technique and gene sequencing.
Results: The results obtained from isolation of *Campylobacters* indicated that out of 70 collected samples, 2 (3%) were positive for *Campylobacter*.
Conclusion: The main way of the entry of these bacteria into the Caspian Sea's water can be thought the household sewages and surface waters pouring into the sea. The existence of the environmental contamination and swage assists noticeably the survival of the species of this bacterium so that they are found more greatly in the contaminated environments. This is because of providing the nutrients for the microorganisms. In 2010, Ghane et al. recorded the first isolation of *Campylobacter* spp. From Caspian Sea's water samples, and the present study and its similar results confirmed that *Campylobacter* spp. exist and survive in the Caspian Sea and previous results was not a transitional contamination.

Keywords: *Campylobacter*, Caspian Sea, Water, Iran

EVALUATION OF BACTERIAL TOLERANCE TO THE ZINC METAL IN THE PHASE 1 INPUT OF WASTEWATER TREATMENT PLANT OF SHAHINSHAHR, ISFAHAN

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Background: Agricultural and industrial activities generate wastewater containing heavy metals such as Zinc, which can be a major problem for the ecosystem and human health. Today, the use of microbial biomass for removal of heavy metals from industrial and agricultural effluents has received special attention. This study was conducted to evaluate the resistance of isolated bacteria from the Phase 1 input of wastewater treatment plant of Shahinshahrto Zinc heavy metal.
Methods: For this purpose, the phase 1 input of wastewater treatment plant of Shahinshahr, sampling was conducted in six plastic bottles and immediately to measure the amount of Zinc heavy metal, BOD, COD, temperature and pH were transported to the laboratory. For isolation of Zinc heavy metal-resistant bacteria, collected serial dilutions from specimens and 0.5 ml of each dilution in the base culture medium metal PHG II, a concentration of 0.5 mM zinc nitrate were cultured in 3 iterations. After isolation of Zinc-resistant bacteria, the minimum inhibitory concentration (MIC) to growth of the bacteria was determined.
Results: Based on the results of Gram staining, all samples were of Gram-positive *Bacillus* and in the most samples, central spore were visible. Then the biochemical tests on two samples of Zinc-resistant bacteria were performed and according to the results of biochemical tests, two species of *Bacillus megatrrium* and *Bacillus sphaericus* were reported and molecular testing is also under consideration. In respect of compatible with Zinc, the maximum tolerable concentration of both *Bacillus megatrrium* and *Bacillus sphaericus*, 12mM was measured.
Conclusion: The isolation and identification of bacteria-resistant to heavy metals will lead to achieving in high efficiency in industrial and agricultural wastewater treatment using bacteria.

Keywords: Bacteria-Resistant, Zinc, Industrial Wastewater Treatment Plant

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A COMPARISON AMONG VARIOUS SELECTIVE MEDIA TO ISOLATE THE NOVEL HEAT RESISTANT ACTINOMYCETES FROM SOIL SAMPLES OF GANDOMBERIAN AREA OF THE LUT DESERT, Iran

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Background: The aim of this study was to evaluate different selective media in the isolation of the heat resistant actinomycetes.
Methods: 46 samples were collected from the surface (surface to 5cm depth) and depth (5-20cm depth) soils of three identified places of Gandomberian (30.34N, 57.51E) in the Lut desert, Iran. The soil sample was mixed, and a suspension of 50 g soil in 50 ml of sterile saline solution was prepared. To selectively isolate the actinomycetes, the suspension heated in water bath at 55ºC for 6 minutes in order to destroy the vegetative forms of bacteria. Four selective culture media (humic acid- vitamin agar containing 50µg/ml cyclohexamide and 10µg/ml nalidixic acid, starch- casein agar containing 25µg/ml cyclohexamide and 25µg/ml nystatin, raffinose- histidine agar containing 25µg/ml cyclohexamide and 25µg/ml nystatin and glucose- yeast extract agar which 20µg/ml rifampicin has been added) were employed. After spreading the samples on the surface of the media, the plates were incubated at 28ºC for three weeks, all of the target organisms were counted as actinomycetes and the results expressed as the number of CFU per gram of soil.
Results: The percent of isolated the actinomycete on humic acid- vitamin agar, raffinose- histidine agar, glucose- yeast extract agar and starch- casein agar, were respectively 61%, 15%, 24% and 39%. The mean and standard error of the isolated bacteria were respectively 7.9±1.2, 0.8±0.3, 1.5±0.5 and 5.5±1.4 CFU/g. No growing was recorded in 12 out of 46 (26.1%) of the samples. The growing rates of the microorganisms on the raffinose- histidine agar were significantly lower than other media ($p<0.05$). Moreover, no significant difference was recorded between the presence of the bacteria in the surface and depth of the samples ($P > 0.05$).
Conclusion: Our results showed that the humic acid- vitamin agar containing 50µg/ml cyclohexamide and 10µg/ml nalidixic acid was the most appropriate selective media to isolate the heat resistant actinomycetes.

Keywords: Lut Desert, Actinomycetes, Humic Acid- Vitamin Agar

EVALUATION AND SELECTION OF ADDITIVES FOR BIOREMEDIATION OF PETROLEUM-CONTAMINATED SOIL USING OIL-DEGRADING BACTERIA

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Background: As the release of petroleum hydrocarbons into the environment cause harmful effects to ecosystem and human life, treating this contamination by applying sophisticated and economical methods such as bio-remediation is necessary and important.
Methods: In this study, additives including bulking agent, nitrogen and phosphorus source were evaluated to find the appropriate additive in order to optimize the bioremediation process in soil contaminated with 5% crude oil which is performed by KE1, K-2-4, MK-1, PM01 and PM06 oil degrading bacterial strains isolated from oil contaminated soils from Kharg Island and the maroon region in Ahvaz. This study was conducted in laboratory and pilot scales. The design of experiments (DOE) was done in Minitab 16. Software by Tagouchi methods and orthogonal array of L18 was designed. In this study, Straw, Rice husk, Diatomaceous earth, Corn cob, Saw dust, Corn shank were selected 5%(W/W) as the bulking agent, NH4CL, Urea, Uric Acid as the nitrogen source and K2HPO4, KH2PO4 and Lecithin as the phosphorous with optimum ratio of C: N: P = 100: 10: 1.
Results: Results revealed that straw for PM06 and K-2-4 strains, corn cob for PM01 and MK-1 strains and diatomaceous earth for KE1 strain were the appropriate bulking agents to provide optimum conditions of bioremediation. About the nitrogen, except PM06 whose proper nitrogen source was urea, for other strains NH4CL showed to be the best nitrogen. About phosphorous source, K2HPO4 for MK-1 strain, KH2PO4 for PM06, PM01, KE1 strains and both KH2PO4 and K2HPO4 for K-2-4 strain were the proper source respectively. For the pilot scale, the bio-reactor with the capacity of 100 kg and 30 x 40 x100 cm dimensions equipped with aeration system was built from the glass of 10mm thickness. Each Bacterial strain was immobilized on the proper bulking agent according to the results of the laboratory scale. NH4CL and KH2PO4 were selected as the nitrogen and phosphorous source with the same ratio as Laboratory scale.
Conclusion: In conclusion, applying bacteria consortia immobilized on a bulking agent will enhance the speed and rate of bio-remediation.

Keywords: Bioremediation,Additive, Oil Degrading Bacteria

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STUDY OF THE PHYTASE PRODUCING BACTERIA EFFECTS ON PHOSPHORUS AVAILABILITY FOR MAIZE PLANTS

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Background: Phosphorus (p) is an essential macroelement for plants and its deficiency is frequent in many regions especially acidic soils. Soil microorganisms play a key role in the dynamic of soil P and can increase its availability for plants by various ways, such as mineralization of organic P using different processes including production and release of organic acids, phosphates and phytase. These bacteria were used as bio-fertilizer since 1950.
Methods: In this research, the efficiency of bacterial strains isolated from different soils for evaluation of phytase releasing potential has been studied. Phytase releasing bacterial strains were isolated from different areas soils around Tabriz and identified using molecular methods. The isolated strains were cultivated on specific culture media (liquid PSM) and their phytase activity was measured at various times. In this study, after isolation and purification of phytase-positive bacteria from soil in specific media, the high efficient strains were selected, proliferated and added to a sterile soil. Experiment was conducted in a completely randomized design and the maize plants were cultivated in the control and inoculated soil. Maize plants were cultivated for 28 days in soil inoculated with phytase releasing bacterial strains, which were isolated from different soils and study of the bacterial effects on growth parameters, dry and weights roots and shoots of maize plants was performed. Chlorophyll concentration was determined using arnun method, Phosphorus concentrations in shoots and roots of maize and Phosphorus concentrations soil were determined using amunium-vanadate method.
Results: Four bacterial strains with phytase releasing potential were isolated from different studied soils but only one strain showed higher potential for phytase production. In general, all of the isolated bacterial strains from different soils can release phytase but the efficiency of strains was different. Results showed that inoculation of soil with selected bacteria increased P availability in soil, increased uptake and transport P, the higher P content in plants shoots and roots, decreased chlorophyll concentrations.
Conclusion: The bacteria *Pseudomonas* sp. Strain2a showed the greatest impact on the availability of soil and Pdecreased chlorophyll concentrations and other parameters in comparison to other strains.

Keywords: Phosphorus Availability, Phosphorus Solubilizing Microorganism, Phytase

EFFECT OF SUGARCANE BAGASSE ADDITION ON THE MICROBIAL COMMUNITY AND DECONTAMINATION OF AN OILY SLUDGE CONTAMINATED SOIL

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Background: During cleaning of storage tanks for crude oil and its derivatives, large volumes of oily sludge are discharged to the environment. Therefore, it can cause environmental contamination particularly soil pollution. One of the bioremediation techniques which are considered more recently is composting. In this method for improving bioremediation process natural and biodegradable bulking agent(s) may be used. Addition of these materials to contaminated soil improves the physical quality of soil and also soil conditioning, therefore enhances microbial degradation and diversity.
Methods: To evaluate the effectiveness of composting on bioremediation of oily sludge polluted soil, laboratory scale bioreactors were designed. Along with the bioreactor which contains polluted soil with sugarcane bagasse (composting) one bioreactor was filled with only polluted soil as the control. Under these conditions the bioreactors were aerated for five months. During this period, changes in culturable bacterial population, oily sludge concentration, and microbial population diversity by using DGGE analysis, were evaluated.
Results: During this analysis, the bioreactor, which contained bagasse, showed enhanced growth of culturable bacterial population and more microbial diversities and oily sludge biodegradation (including oily sludge weight loss). Organic compounds soluble in toluene, saturated hydrocarbons and asphaltenes decreased too.
Conclusion: Bioremediation of oily sludge contaminated soil by composting effects on total number and diversity of microbial community of soil and this result in more bioremediation of oily sludge contaminated soil

Keywords: Bioremediation, Composting,Sugarcane Bagasse, DGGE Analysis

ISOLATION OF PIGMENT-PRODUCING BACTERIA FROM SURFACE WATER AND STUDY OF SUN PROTECTION FACTOR (SPF) OF THE PURIFIED PIGMENTS

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Background: The aims of this study are isolation of pigment-producing bacteria from surface water and study of Sun Protection Factor (SPF) of the purified pigments.
Methods: Ten surface water samples were obtained from different area of tonekabon-chalus cities. Each of samples was transferred into marine broth medium followed by incubation at 30°C, 180 rpm for 24 h. A 10-fold serial dilution of each sample was prepared while 100 µL of 10^{-5} and 10^{-6}dilution was plated onto marine agar. The isolated colonies were repeatedly streaked to obtain pure cultures.The pigment extracted by ethanol solvent. The in vitro SPF number was determined according to the spectrophotometric method described by Mansur et al.
Results: Three pigments were selected among other extracted pigments. The SPF number of bacterial pigments was calculated by applying Mansur mathematical equation. The extracted pigments are orang pigment (extracted from coccobacilli gram positive), red pigment (extracted from bacilli gram negative) and pink pigment (extracted from coccobacilli gram negative), SPF value of the pigments were found to be 1.785,1.629 and 2.72, respectively.
Conclusion: The efficacy of a sunscreen is usually expressed by the Sun Protection Factor that is a useful assessment of primarily UVB (290-320 nm) filters. An SPF of 15 means that if it takes 10 minutes for skin to start to burn without sunscreen it will take 150 minutes with that sunscreen.

Keywords: Pigment,Bacteria,Extraction, SPF

ISOLATION AND IDENTIFICATION OF ENVIRONMENTAL HALOPHILIC BACTERIA PRODUCING INDUSTRIALLY IMPORTANT ENZYMES FROM SALTY SOILS

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Background: Industrially important enzymes are endowed with catalytic power to sustain microbial metabolic and physiological processes under salt conditions. The aim of the study was isolation and derivation of halophilic bacteria producing enzymes of industrial value.
Methods: One gram of the collected salty soil samples was weighed and dissolved in 9 ml of sterile distilled water. Serial dilution was done up to 10-5 and cultured on a semi-differential medium supplemented with 10% NaCl using pour plate method and incubated at 30 °C for 72 h. The pure culture of the bacterial isolates was obtained via sub-culturing. To evaluate the activity of amylases, proteases and chitinases, isolates were inoculated onto NA medium supplemented with starch 2 % (w/v), skim milk 1 % (w/v) and colloidal chitin 1 % (w/v) respectively. The colonies showing a clear or dim halo were picked as positive exoenzyme-producing isolates. The identity of isolates were studied morphologically, biochemically and molecularly. Molecular identification of the selected strains was done by amplification, sequencing and NCBI-BLAST comparison of 16S rDNA sequences employing universal primers 27F (AGAGTTTGATCCTGGCTCAG) and 1492R (ACGGCTACCTTGTTACGACTT).
Results: Initially, 30 isolates were found to be halophilic tolerating NaCl concentrations ranging from 2.5-12.5%. From these, 25 protease positive, 6 chitinase positive and 12 amylase positive strains had immense potential for enzyme production. The identification studies finalized by BLAST comparisons and phylogenetic analyses revealed that the chosen isolates belonged to the genera of *Streptomyces* and Nocardia.
Conclusion: This study reassured that salt rich lands accommodate bacterial strains capable of producing hydrolytic enzymes such as protease, chitinase and amylase. A local collection of industrial enzyme producing domestic bacterial isolates is now in hand which paves the way for further research into optimizations and enhancements.

Keywords: Protease, Detection, Microorganism Halophiles, Protease, Amylase

ASSESSMENT OF ENZYMATIC ACTIVITY IN MARINE ACTINOMYCETES

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Background: To evaluate the enzyme activity of actinomycetes isolated from the Persian Gulf, as untapped source for searching industrial enzyme.
Methods: A total of 20 marine samples were collected from the some northwest region of Persian Gulf. Isolation was done using Starch casein agar and ISP2 agar (Yeast extract - malt extract agar). Amilase, protease, gelatinase, lipase, caseinase, cellulase, Dnase and chitinase enzymes produced were evaluated using the respective medium. Identification of 3 strains of the best producers was carried out by morphological, physiological, chemotaxonomic and biochemical methods.
Results: 30 strains of actinomycetes were isolated from samples. About 60% of strains were able to produce enzymes. MW5, MW4, SS2, SSC5 and BW5 strains were identified as *Streptomyces* sp using Shirling and Gottlieb 1966 with Bergeys manual of determinative bacteriology.
Conclusion: Marine actinomycetes are the most potent industrially important the microorganisms which are capable for the produce bioactive compounds like enzymes. The current investigation reveals that the marine actinomycetes from the Persian Gulf could be vital sources for the discovery of industrially useful molecules and enzymes.

Keywords: Marine Actinomycetes, Enzymes, Persian Gulf

PHYSIOLOGICAL CHARACTERIZATION OF CYANOBACTERIAL POLYSACCHARIDES

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Background: Todays, cyanobacterial polysaccharides have found a new position among the biotechnological applications. These water-soluble polysaccharides that produced by microorganisms particularly cyanobacteria, attracting interest as they have great application potential in food, cosmetic, pharmaceutical and oil industries, as thickening, stabilizing and emulsifying agents. Therefore, in this research comparative study and characterization of cyanobacterial polysaccharides production carried out, for the first time in Iran.
Methods: Cyanobacterial samples were chosen from various orders as follow: *Nostoc* sp. (ISC 101) (filamentous with heterocyst), *Phormidium* sp. ISC108 (filamentous without heterocyst) and *Synechococcus* sp. ISC 106 (unicellular). The first one was cultured in BG110 (without combined nitrogen) but the others were in BG11 medium (Rippka et al., 1979). Growth rate and curves were studied by Chlorophyll a extraction in methanol (Marker, 1972). Polysaccharides were estimated by colorimetric method (Dubois et al., 1956).
Results: Growth of specimens was estimated more than a month. During this period, Nostoc sp. had the highest growth content. The sugar contents of all samples had increasing rate after 30th day but the unicellular sample (Synechococcus sp.) had the highest rate among the two other samples.
Conclusion: After a month, production of cyanobacterial polysaccharides increases significantly. The unicellular specimens like Synechococcus sp. produce the highest amount of polysaccharides and then Phormidium sp. has the second rate.

Keywords: Characterization, Physiology, Cyanobacteria, Polysaccharides.

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BIOLEACHING OF MANGANESE FROM STONE WHICH CONTAINS PYROLUSITE THROUGH NATIVE MICROBIAL STRAINS IN Iran

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Background: Bioleaching of manganese is an industrial method for its recovery from stone which contains pyrolusite (Mno2). In this method, the host minerals are analyzed and revived by certain bacteria. The present research has been done in Iran, for the first time, to study the bioleaching of manganese from stone which contains pyrolusite.
Methods: Different samples of soil & water were collected from different mines of manganese in different regions of Ian. LB medium and Brunner were used to isolate and enrich microorganisms, and then the action of purifying and identifying grown microorganisms performed through biochemical methods. The bioleaching of manganese was done in flask during 30 days, under the best conditions of temperature, aeration and the diameter or thickness of articles in order to select the best conditions and the highest strain. The amount of revived manganese was measured by two methods of spectrophotometric and titration.
Results: In the present research, 13 bacteria which revived manganese were isolated including *Bacillus*, *micrococcus*, *Corynebacterium*, *Staphylococcus*, *Serratia*, *Lactobacillus*, and *Enterobacter* from CheshmeKabud mine in Kermansah. The results of Pyrolusite mineral bioleaching with particle diameter of 75 microns, under heat treatment of 30°C and moving speed of 180 rpm, during 2,10 and 30 days indicated that the highest revival of manganese from pyrolusite mineral performed after 30 days to the amount of 33% by Mg4 *Bacillus* bacteria strain.
Conclusion: The results obtained from the present investigation indicated that the strains of *Bacillus* genus have the highest power of reviving in bioleaching of manganese from pyrolusite mineral. Also, with the increase of time, the percentage of bioleaching increased in all bacteria in comparison with control ones.

Keywords: Bioleaching, Manganese, Pyrolusite, Native

EFFECT OF SALT RESOURCE ON THE HALO-BACTERIUM SALINARUM GROWTH IN FED-BATCH CULTURE WITH AERATION

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Background: Halophilic Archaea based on structural characteristics, physiological, biochemical and genetically distinct from other microorganisms has attracted the attention of scientists. *Halobacteriumsalinarum* produces a membrane embedded photoprotein with several proposed applications in biotechnology. However, low growth rates of halophilic archaea, often act to hamper all further biotechnological advances. Therefore, to achieve a high rate of growth, culture medium engineering could be affective. One main component of the medium is sodium chloride that its resource and purity may affect the microorganism growth. In this research we compare the effect of two types of the salt on the *H. salinarum* growth rate.
Methods: *H. salinarum* R1 cultured in a designed reactor with inlet and outlet air tubethat placed in the Shaking incubator at 39 °C and 250 rpm. Air provided by a compressor that pumped the filtered stile air through a humidifier into bottom of reactor and exhausted from outlet air tube. Humidifier temperature was equal with growth temperature. Two kind of salts used in this study; first one was purified salt via crystallization procedure. The other one was non purified sea salt from Urmia salt lake. Feeding performed every day and concentration of feeding culture medium components except salt increased depending on the optical density of the microorganism at 600nm.
Results: After 100 hours, OD600 of the microorganism grown in the Medium containing non purified sea salt was as much as 8 whereas OD600 in the case of purified sodium chloride was 4.78 at the same time.
Conclusion: It seems that the impurities of the sea salt may have some trace elements that improved aerobic growth of the *H. salinarum*R1.

Keywords: Halophilic Archaea, *H. Salinarum*r1, Fed-Batch Culture Non-Purified Sea Salt

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PREVALENCE OF BACTERIAL CONTAMINATION IN WATERLINES OF HAMADAN DENTISTRY SCHOOL UNITS AND DRINKING WATER SUPPLY OF LOCAL AREA

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Background: The purpose of this study was to evaluate the bacterial contamination and biofilms formation of waterlines of dentistry units at dental faculty of in Hamadan University of Medical Sciences and drinking water sources contamination of Hamadan city.
Methods: In this cross-sectional study, overall 114 internal and external water samples from dental units in the different department of dentistry faculty and simultaneously 10 drinking water samples of city were selected to assess microbial contamination in water sources. Samples were taken based on active dental units and divided for 6 units at 3 times in the working day in (8, 10 and 12 o'clock of day), 50 ml of water sample were taken before flashing and 50 ml after flashing were collected in sterile polyethylene container. Samples were transported in closed sterile containers to microbiology laboratory of Hamadan University of Medical Sciences and cultured in convenient media cultures. Colony count forming units and bacterial isolated were also detected by differential tests. Data was gathered through a questionnaire and analyzed using Men-vitni and SPSS 16 software.
Results: Of 114 cultured samples from waterlines of dentistry units, 41 positive cultures (35.9%) were obtained. From 41 positive cultures, 21 bacteria species and fungi (51.2%) were isolated. The most important gram-positive bacteria were as follow: *Micrococci leutus* (47.6%), and *Staphylococcus epidemidis* (28.6%), and 20 gram-negative bacteria (48.8%) were isolated that he most important gram-positive bacteria were as follow: *Brevundimonas* (35%), *Acinetobacter bummani* and *Pseudomonas aeroginosa* (15%). From 41 bacteria species isolated of waterlines of dentistry units, 15 isolates (36.5%) from internal water and 26 isolates (63.5%) from external water were isolated. The mean of colony count forming units (CFU) from internal water was 610 CFU/ml and 1264.1 CFU/ml for external water of dental units. From 56 water samples that were taken from wards of Endo, Pediatrics, Periodontal, Surgery and prosthodontic., overall 15 biofilms (26.7%) were obtained, From 10 drinking water samples of city, only 1 species (10%) incuding *Micrococci leutus* was isolated
Conclusion: Our results showed that bacterial contamination of external waterlines was relatively more than internal waterlines from tested dental units. External waterlines were contaminated with *Pseudomonas aeruginosa*, however, coliforms were not observed.

Keywords: Bacterial Contamination, Water, Dentistry Unit, Biofilm

INVESTIGATION OF THE ACETAMIDE DEGRADATION BY A BACTERIAL STRAIN, SH35, WHICH ISOLATED FROM WATER SAMPLES IN KERMAN JAME'A MOSQUE

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Background: The purpose of this study was evaluation of acetamide degradation by native bacteria.
Methods: The water samples were collected from various area of Jame'a Mosque, Kerman. For the screening of acetamide degrading bacteria, species were cultivated on mineral salts medium (MM1) agar media containing acetamide (25 mM) and phenol red (2.5 %), as pH indicator. The ammonia released was measured at different time incubation by Bertholet reaction.
Results: Among 40 isolated species, Sh35 was selected as a better species in degrading acetamide. This strain shows a significant pink halo against yellow background on MM1 agar supplemented with acetamide and phenol red.This species was belonged to *Bacillus* genus based on biochemical and molecular tests. Results show that amidase activity of this strain was highest after 24 h incubation in the liquid medium.
Conclusion: Amide degradation capacity of this strain suggested that, it might be suitable for the bioremediation of hazardous compounds.

Keywords: Nitrile, Acetamide, Bioremediation, Screening

TAXONOMIC CLASSIFICATION OF NEWLY ISOLATED ARCHAEAL STRAIN FROM URMIA HYPERSALIN LAKE

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Background: Urmia Hypersaline Lake which is located in the Northwest of Iran has been explored with great extreme haloarchaeal diversity in recent years. Extreme halophilic Archaea are widely distributed in hypersaline habitats such as salterns and lakes. During the course of biodiversity studies in Urmia Lake, we have isolated several extreme halophilic Archaea some of which could represent as new taxa.
Methods: Among these strains, we chose strain DA50 for further characterization. As our studies demonstrated, this strain showed 16S rRNA gene sequence similarity with Natrialba aegyptia 40T (92.9%). We also performed some biochemical, morphological and physiological tests for aforementioned strain according to minimal standards in order to characterize the isolate.
Results: Cells of this strain were pleomorphic. This microorganism was VP negative, MR positive and catalase positive. Profile of DA50 for acid production, and hydrolize of galactose, sucrose, starch, fructose, lactose, ribose, mannitol, glycerol, maltose and glucose was analyzed. Furthermore, using these sugars as sole source of carbon in its metabolism has been surveyed. Strain DA50 showed optimum growth at 22 NaCl% (w/v), optimum pH for the strain growth was 7 and optimum temperature for growth was between 40 to 50 °C. This strain was also unable to hydrolyze starch, DNA, tween 40, tween 60 and tween 80. According to antibiogram test results, DA50 was resistant to cephoxitin, amikacin, carbenicillin, streptomycin, nalidixic acid, tobramycin, cephalotin and penicillin while it showed susceptibility to some other antibiotics such as rifampicin, baciteracin and amoxyclave.
Conclusion: Based on obtained data, this strain has 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 this strain as the member of new taxa in genus level.

KeywordsHaloarchaea, Polyphasic Taxonomy

EFFICIENCY OF LACTIC ACID BACTERIA IN BIOLOGICAL CONTROL AGAINST FUSARIUM SP. IN CORN FIELD IN GORGAN AREA

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Background: Soil-borne fungi such as Fusarium are plant pathogens like cereals and other crops. Since disabling this, funges and their produced mycotoxins by physical and chemical methods are costly and destroy nutrients, so biological detoxifications used to neutralize them and since lactic acid bacteria capable of produce antifungal metabolites, they are used as biocontrol agents.
Methods: A sample of wheat isolated from 10 store rural areas in Gorgan during two season the winter and spring and identification of fusarium fungi was done based on morphological features and keys for the identification in culture media PDA and CLA. Then their growing the inhibitory effects was studied by 7 strains of lactic acid bacteria with two-layer cultured and diffusion well in vitro.
Results: A total of 60 collected wheat samples,29 strains of *Fusarium* fungi isolated (48%) and of this number, *F.verticillioides* was the most common. The Results of survey inhibition effect of lactic acid bacteria as well method shown that the bacterium *Lactobalillus rhamnosus* had highest inhibitory effect on fusarium species. *L. Lactis* had minimum inhibitory effect on Fusarium species have been isolated. *Lactobalillus rhamnosu*s had been only effective bacterial on F.verticillioides. Maximum and Minimum Inhibitory effect has been against pathogenic fungi F.solani and *F.geraminearum*, respectively. After 48 h incubation, the biggest change in the diameter has been in Fusarium solani.Because of Fusarium solani is ahuman pathogen, therefore two-layer cultured performed only for it.
Conclusion: Results demonstrated that the lactic acid bacteria on isolated Fusarium fungi were well more effective than two-layer culture. So many families of bacteria lactic acid capable of producing material to prevent growing pathogenic fungi, therefore they are good choice for the inhibition of the Fusarium Fytopatogeny fungus.

Keywords: Biological Control, *Fusarium*, Acid Lactic Bacteria

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THE EFFECT OF FOUR DIFFERENT CHEMICAL VEGETABLE DISINFECTANTS AGAINST FOUR FOOD-BORNE PATHOGENS

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Background: It is important to prevent bacterial transmission from fresh produces because of increasing cases of food-borne diseases especially due to vegetables. The purpose of this study was to determine the effect of four approved vegetable disinfectants on three concentrations of food-borne bacteria.
Methods: Four food-borne bacterial strains (*E. coli, Staphylococcus aureus, Listeria monocytogenes and Salmonella typhimurium*) were provided from bacterial collection of scientific and industrial research organization and adjusted in three different concentrations (103,105 and 107 CFU/g). Four disinfectants (Benzalkanium chloride, Chlorine, Hydrogen Peroxide+Ag+ and Peroxy Acetic Acid) were diluted based on user instruction. Inoculation was done and studied in three different time durations near user instruction for each disinfectant (10, 15 and 20 minutes). Two-way ANOVA was selected as statistical method.
Results: All four disinfectants were able to reduce count of all four bacteria from10^3 and 10^5 CFU/g to acceptable standards according to concentration referred in each disinfectant user instruction. But in 10^7 CFU/g, only benzalkanium chloride could be effective. All four disinfectants could reduce count at time exposure equal or more than time mentioned in instruction. Only b.c. was effective at time lower than instruction.
Conclusion: All disinfectants were able to reduce bacterial count 5 log at time exposure mentioned in user instruction. If the contamination was higher, only one of them could be effective. It is suggested to emphasis the consumers to wash all fresh vegetables at least one time before disinfecting to decrease initial microbial load.Also time is important for all disinfectants.

Keywords: Vegetable, Bacteria, Contamination, Disinfectant.

ANTIBACTERIAL ACTIVITY OF TARRAGON (ARTEMISIA DRACUNCULUS) ESSENTIAL OIL

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Background: Nowadays, adding natural food preservatives is one of the methods for increasing shelf-life. The aim of this study was evaluation of antibacterial effects Tarragon (Artemisia dracunculus) essential oil (TEO) in beef burger product.
Methods: In this experimental study, essential oil of the Tarragon was isolated by hydrodistillation. Then, TEO was analyzed by gas chromatography-flame ionization detector (GC-FID) and gas chromatography/mass spectrometry (GC-MS). The effect of different concentrations of Tarragon essential oil (0.00, 0.062, 0.125 and 0.25%) in 4±1ºC temperature and storage time up to 12 days was evaluated anti *Staphylococcus aureus* activity in beef burger.
Results: The monoterpenes hydrocarbons constitute the major fraction of the TEO (95.91%) and the sesquiterpene hydrocarbons were the minor fraction (0.46%). The minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) for *Staphylococcus aureus* were between 62.4to 250 mg/mL, respectively. The Tarragon essential oil 0.25% in storage temperature (4±1ºC) decreased growth rate of *S. aureus* in beef burger ($p<0.05$).
Conclusion: Therefore, this essential oil might be used as an antibacterial agent in meat products such as beef burger.

Keywords: Antibacterial Effect, Artemisia Dracunculus, Beef Burger

EVALUATION OF AMYLOLYTIC ACTIVITY OF LACTOBACILLUS ISOLATES FROM TRADITIONAL SOURDOUGHS

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Background: Lactic acid bacteria are widely used in the food industry as starter cultures. Acidification of the dough, proteolysis of gluten and moderate hydrolysis of starch are lactic acid bacteria metabolic activities, which affect bread quality. Some lactic acid bacteria have amylolytic activity and possess amylases to utilize starch for lactic acid production. Amylolytic lactic acid bacteria have been isolated from fermented foods like sourdoughs.
Methods: In this study, amylolytic activity of 71 lactobacilli isolated from traditional sourdoughs was evaluated in two types of starch agar media.
Results: The results showed that the medium containing rice starch was more suitable for evaluating amylolytic activity of *Lactobacillus* isolates. Sixty five *Lactobacillus* isolates had amylolytic activity and were able to growth in starch agar medium containing rice starch
Conclusion: The *Lactobacillus* isolates 9, 15, 26, 29, 38, 49, 68 and 51 had a good amylolytic activity.

Keywords: Amylolytic Activity, Lactic Acid Bacteria, *Lactobacilli*, Sourdough

URMIA HYPERSALINE LAKE: A POTENTIAL SOURCE OF ACTINOMYCETES POSSESSING

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Background: The aim of this study was to discover new natural products from novel actinomycetes isolated from unexplored Urmia Hypersaline Lake.
Methods: Marine samples were collected from different points in the coastal of Urmia lake and totally 20 actinomycetes were isolated using different isolation media. All the isolates were characterized and identified by microscopical and macroscopical observations. Sequencing and analysis of 16S rDNA from chosen isolates was performed.
Results: Identification of the isolates revealed that all isolates belong to the genus *Streptomyces* sp. The isolated marine actinomycetes were screened for their antimicrobial activity against the human bacterial pathogens *Salmonella typhi, Escherichia coli, Bacillus cereus*, *Staphylococcus aureus* and *Klebsiella pneumonia*. Among 20 isolates, 8 showed antibacterial activity. The marine isolate *Streptomyces* sp. CS1 was found to be more efficient in the production of secondary metabolites.
Conclusion: In conclusion, escalating reports on discovery of diverse natural compounds from halophilic and halotolerant actinomycetes, which inhabit in Urmia Lake, suggested that this physiological group has enormous capacity to produce array of secondary metabolites with disparate activities.

Keywords: Antibacterial Compounds, Extreme Environments, Actinomycetes

EFFECT OF WHEY PROTEIN BASED EDIBLE COATING ON THE MICROBIAL PROPERTIES AND TOTAL VOLATILE NITROGEN OF FRESH MUTTON

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Background: The main objective of this research was to investigate the effect of whey protein based edible coating on the microbial properties and total volatile nitrogen of fresh mutton.
Methods: Coating solution was prepared according to McHugh et al.After the coating solution formation, twelve samples were randomly selected and followed by immersion in solution (1min). The Samples were then kept overnight in refrigerator for 1, 3 and 5 days at storage conditions with exposed surface area (23 ± 2°C, RH 50%).The microbial properties (total count and psychrophilic bacteria) and Total volatile of Nitrogen of the coated and uncoated samples were analyzed.
Results: The results obtained from variance analysis showed no significant differences in total count, psychrophilic bacteria and total volatile nitrogen of the coated and uncoated samples ($p>0.05$).
Conclusion: The results of this study showed that the effect of whey protein edible coating on the microbial properties and total volatile nitrogen of meat during 0, 1, 3 and 5 days storage conditions had no significant different ($p>0.05$). The barrier of protein coating is affected by water absorption. Therefore, the relative humidity (RH) can substantially affect coating properties.Therefore; the inclusion of a preservative in an edible coating enrobed on to the product positions the preservative at the point of the food's greatest susceptibility to deterioration.

Keywords: Coating, Mutton, Microbial, Total Volatile Nitrogen, Whey Protein

SUBSPECIES-SPECIFIC IDENTIFICATION OF BIFIDOBACTERIUM ANIMALIS SUBSP. LACTIS USING 16S-23S RIBOSOMAL RNA INTERGENIC SPACER (ITS) GENE

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Background: The purpose of the study was to use ITS region for molecular identification of *Bifidobacterium animalis* subsp. lactis.
Methods: Human fecal samples were collected. All samples were cultivated in MRS agar and Morphological assessments of colonies were confirmed by gram staining. Suspected microorganisms were subjected for genomic DNA extraction. 16S-23S ribosomal RNA intergenic spacer gene from *Bifidobacterium animalis* subsp. lactis was chosen for primer design based on Primer-BLAST online tool of NCBI data base and PCR assay were setup on the genomic DNA. Final approval of the assay was performed with sequencing of PCR product.
Results: Genomic DNA amplification of samples along with the 16S-23S ribosomal RNA intergenic spacer gene primers exhibited the presence of 226 bp band, as expected. Sequencing data analysis was performed by BLAST software of NCBI data base. The results of BLAST showed 100% homology of its DNA sequence with the sequence obtained from Genbank for *Bifidobacterium animalis* subsp. lactis. The sequences are deposited in the Gene Bank with the accession number: KJ558387.
Conclusion: Specific identification of lactobacili species and subspecies with microbiological tests are laborious and time-consuming. Molecular techniques can be replaced by microbial tests. The rRNA gene (rDNA) has been used widely to infer phylogenetic relationships among bacteria. However, as evolutionary distances decrease, the diversity found in the 16S rDNA is often insufficient and genetic relationships of closely related species cannot be accurately defined. The results of the present study demonstrated that PCR amplification of 16S-23S ribosomal RNA intergenic spacer gene is the appropriate test for subspecies-specific identification of *Bifidobacterium animalis* subsp. lactis.

Keywords: *Bifidobacterium lactis*, Probiotic, ITS Gene, 16S-23S Ribosomal RNA, Spacer