Resuscitation of Viable but Non-Culturable Escherichia Coli, Using Environmental Shocks

Heiat M, Abbasi Larki R*, Aghamollaei H, Mousazadeh Moghaddam M, Yaali Jahromi A
* Reza_abbasi.biology@yahoo.com

ABSTRACT

Background and objective: The lack of bacterial growth on media culture is not always a reason of the non-existence or death of the bacteria. Being alive, some bacteria can obtain a status in which they lose their growth ability on the common media culture. The status of the bacteria survival is defined as Viable but non-culturable those only the tests based on bacteria survival confirm their viability and other tests are unable to diagnose them. This status can be a serious factor to outbreak some infectious diseases caused by the use of some materials of which the microbial safety is approved. applying some environmental stress, the present study investigates Non-culturable E. coli ability to dispossess from this phase and being resuscitated.

Materials and methods: After collecting several samples, Non-culturable E. coli were divided into different groups in order to carry-out different experiments. They were liking to heat shock of 42ºC in different periods of time, different concentration of Bile-salts and NaCl and combinational of these methods.

Results: 15 mM salts, 42 mM NaCl, and 42ºC heat shock within 2 min $1x10^4$,$0.6x10^4$ and $3/1x\ 10^4$ CFU/ml respectively. The combination of these parameters in a binary manner, also inferred to suitable results. Applying the three stresses simultaneously arising optical density up to 0/58 and $9x10^8$ CFU/ml presented the best respond.

Conclusions: The results show that applying some alterations in the condition of bacterial culture, the growth path of the bacteria which remain to a stationary phase can be changed to the normal status.

Key words: Non-culturable bacteria, E. coli, Bile-salts, NaCl, Thermal Shock.

Distribution of bla_{SPM-1} and bla_{GES-1} Genes among Imipenem-Resistant Acinetobacter spp.in Tehran, Iran

Abbasali Pourbashshash M. Shahcheraghi F*
*****shahcheraghifereshteh@yahoo.com

ABSTRACT

Background and objective: High intrinsic and acquired resistance of Acinetobacter infections has caused the problem. One of the main mechanisms of resistance toβ- lactam antibiotics are beta-lactamase production. This study aimed to determine the antibiotic susceptibility pattern and investigate the presence of beta-lactamase genes bla_{SPM-1} and bla_{GES-1}among clinical isolates of **Acinetobacter**isolated from the Iranian patients at Tehran hospitals.

Materials and methods : Antibiotic susceptibility profile for 100 imipenem resistanceclinical isolates of Acinetobactr collected from Tehran hospitalswas determined by the disk diffusion method and then MICs of imipenem were detected using Micro broth dilution (CLSI). PCR was performed for detection of bla_{SPM-1}, bla_{GES-1}carbapenems.

Results: Among203 samplestested, 100 strainsresistant toimipenemwere isolated. Antimicrobialsusceptibilityresultsindicate Multi-Drug-Resistance among isolates.Our results showed that 100% studied isolates showed MIC≥8µg/ml to imipenem. bla_{SPM-1}, bla_{GES-1}were detected among 6%,2%, isolates respectively.

Conclusion: Betalactam-resistant Acinetobacter strains are increasingly recovered from hospitalized patients worldwide and our results obtained from clinical isolates of Acinetobacter showed high resistance to betalactam antibiotic as well. Understanding the underlying genetic mechanisms mediated antibiotic resistance could eventually facilitate the development of effective prevention and control strategies and thereby allowing more effective drug usage and treatment of disease, and reducing resistance development.

Keyword: Acinetobacter, Antibiotic resistance, SPM, GES

Multidrug-Resistant (MDR) ESBLs Producing Diarrheogenic E. coli Strains Isolated from Pediatric Patients

Ghorbani-Dalini S*, Kargar M, Doosti A, Golshan M, Sarshar M
* ghorbanidalini@jia.ac.ir

ABSTRACT

Background and objectives: The emergence of MDR and extended Spectrum-β-lactamases producing Escherichia coli poses antibiotic management problems. ESBLs are cephalosporinases that confer resistance to a wide variety of oxyimino cephalosporins and create serious therapeutic problems. The aim of this study was to assess the prevalence of bla_{TEM}, bla_{SHV} and bla_{CTX-M} genes in diarheagenic E.coli isolated from children less than 5 years of age.

Materials and methods: In a cross sectional study, 77 E. coli strains isolated from patients with diarrhea in Shiraz was included for the investigation. E.coli strains were isolated by common biochemical analysis. Disk diffusion and DDS analysis were performed for antibiotic resistance analysis. The occurrence of ESBLs (bla_{TEM}, bla_{SHV} and bla_{CTX-M}) producing isolates was studied according to CLSI guidelines and the genetic nature of ESBLs resistance was investigated by PCR.

Results: Antibiogran analysis revealed that penicillin and macrolid resistance were more common and carbapenem resistance is rare. Multi-drug resistance (MDR) was shown in 59 (76.62%) strains. Out of 77 samples, ESBL-related bla genes were detected in 72 (93.50%) isolates which were single bla_{SHV}, bla_{SHV} and bla_{CTX-M} genes identified in 40, 3 and 1 strains and multiple genes identified in 28 isolates.

Conclusions: Our results showed that, unlike other parts of the world, bla_{TEM} was the most common gene in this study. The high prevalence of multidrug-resistant strains, emphasize more attention to the administration of appropriate antibiotics for treatment of E. coli related disease.

Key words: MDR, Diarrheagenic E. coli, Children, bla_{SHV}, bla_{SHV}, bla_{CTX-M}

Expression of Surface Protein (SAG3) Gene of Toxoplasma gondii in Eukaryotic Cell

Sobati H, Dalimi A*, Kazami B, Ghaffarifar F
* dalimi_a@modares.ac.ir

ABSTRACT

Background and Objective: Toxoplasma gondii is the intracellular protozoan parasite responsible for animal and human toxoplasmosis. SAG3 posses an important role in attachment to target cells. SAG3 antigen is expressed at different parasite life stage such as tachyzoite, bradyzoite and sporozite . Regarding these specifications it can be used as a candidate for vaccine and disease diagnosis. The aim of this study was the expression of the gene which encodes the complete surface protein3(SAG3) of T.gondii in CHO cell.

Materials and methods: In the present study SAG3 gene was cloned into pBluescript plasmid following amplification by PCR, and then subclone into pcDNA3 plasmid which was incised by BamHI and HindIII enzyme. Finally the recombinant plasmid (pcSAG3**)** was transfected into CHO cell for expression and the resulted product was confirmed by RT-PCR ،SDS-PAGE and Western blot analysis.

Results: The SAG3 gene was cloned successfully into pcDNA3 vector by using the restriction enzyme HindIII and BamHI. Extracted plasmid was confirmed by PCR and enzymatic incision and sequencing. SAG3 was synthesized in a eukaryotic system successfully. The CHO(transfected and non transfected control cells) was harvested for 48-72h following the transfection. The protein and RNA extracts were then analyzed by SDS-PAGE ,Western blot and RT-PCR. A band at about 43kDa was recognized by Western blot using Toxoplasma antibody-positive human sera and 1158bp band in RT-PCR. SAG3 protein was not detected in nontransfected control cells.

Conclusion: We successfully cloned the complete SAG3 gene into expression plasmid pcDNA3 and expressed it by eukaryotic cell. The expressed SAG3 can be used as a good candidate for recombinant vaccines and also to produce a diagnostic kit.

Key words: Toxoplasma gondii, SAG3 gene, cloning, expression, cell eukaryotic

Multiplex PCR for Rapid and Specific Detection of Shigella flexneri 2a

Tat M, Saadati M, Hosseini M*, Kamali M, Keihan AH, Hosseini Ataei M, Koushki H, Rashidiyani J, Hesaraki M
*Geneticman2005@gmail.com

ABSTRACT

Background and objective: Shigellosis or acute bacillary dysentery as major of cause's infection is prevalent in Iranian children that suffer from diarrheal. Shigella flexneri serotype 2a is considered as a significant concern for public health in developing or developed countries, which due to the role of this pathogen in a shigellosis endemic and mortality. In current study, a multiplex PCR (mPCR) reaction, with purpose of rapid and specific detection of Shigella flexneri 2a from shigella's stool separated which isolated from patient with shigellosis, was designed and evaluated.

Materials and methods: A total of 27 isolated of shigella flexneri were collected from children with acute bacillary dysentery or gastroenteritis (inflammation digestion system), who were admitted to Taleghani, Milad and baqiyatollah hospitals during January up to July 2011. Using bioinformatics analysis with Shigella genome sequences, we designed the flex2a-F and flex2a-R primers, which exclusively amplify specific island (she) in Shigella genome. After extraction genomic DNA, in order to identify presence serotype 2a and to determine specifity and sensitivity, the mPCR as differential method was done.

Results: By use of biochemical and serological tests, the presence of the shigella's species in the all of the hospital's samples was verified. The results of bioinformatics analysis demonstrated the region with length of 1697 bp in shigella flexneri 2a, was exclusively specific. The results of mPCR confirmed 4serotype (19.04%) of hospital's samples was belong to shigella flexneri 2a. The specifiy of mPCR (100%) was determined. The reaction's sensitivity detected 85 cfu (colony forming unit).

Conclusion: The she region on shigella genome, as particular region for identification present of serotype 2a in hospital's samples is specific. Furthermore, the mPCR method as valuable tool in epidemiological studies is more suitable and reliable.

Keywords: Shigellosis, Shigella flexneri 2a, Multiplex PCR, Bioinformatics.

Biofilm in Urinary Tract Pathogens

Rahmani G, Mohammadi P*, Abdi-Ali A
*p.mohammadi@alzahra.ac.ir

ABSTRACT

Background and objective: Today, urinary catheters are one of the most important and medical devices used in different wards in hospital and the prolonged use of them can result in serious risks of blood infection and mortality. The aim of this study was to be characterizing pathogenic bacteria from the surface of catheters and assay their ability in the production of biofilm.

Methods and materials: In this research 50 catheters from patients in ICU ward of Army Family Hospital (Tehran) were studied. Catheters were transported to the laboratory using of sterile buffer. Isolated bacteria were identified by biochemical tests and biofilm formation was measured quantitatively by microtiter plate and crystal violet method.

Results: In this study different percentage of bacteria and yeast were isolated and identified which included gram negative bacteria: %19.44Escherichia spp, %12.96 Acinetobacterspp, % 6.48 Pseudomonas spp, %4.62Klebsiellaspp and Enterobacterspp 1.85 as wellincluded gram positive bacteria: %19.44 Enterococcusspp and %10.18 Staphylococcus spp and % 25 yeast. The assayof biofilm formation in this bacteria showed that about 84% of the isolates can produce biofilm and Pseudomonas spp and Acinetobactersppcan produce stronger biofilm

Conclusion: It can be concluded that many of bacteria which can produce more and stronger biofilm are gram negative and belong to Pseudomonas sppand Acinetobacterspp that are perhaps related to specific outer structure.

Keyword: biofilm, urinary catheter, urinary infections

Antibiotic Resistance Pattern of Helicobacter Pylori

Sayyadi S*, Darbouei M, Dabiri H, Shokrzade L, Mirzaie T, Albouye M, Nazemhossini E, Zali MR
***** ara_sayyady@yahoo.com

ABSTRACT

Background and objectives: Helicobacter pylori are an important cause of human gastric in developing countries. Triple therapy; a proton pump inhibitor, metronidazole and amoxicillin, or clarithromycin are used worldwide for treatment. By adventing antibiotic resistant strains, the treatment has become complicated. The current study fallows Helicobacter antibiotic susceptibility trend to major antiobiotics during 2009-2010.

Mterial and methods: gastric biopsies from patients with dyspepsia were cultured on H. pylori specific media with microareophilic condition. All strains were confirmed as H.pylori by catalase, urease, and oxidase test. PCR for glmM gene were used for confirmation. Antibiotic susceptibility testing for clarithromycin, tetracycline, amoxicillin, metronidazole and ciprofloxacin were performed according to CLSI guidelines by agar dilution method.

Results: out of 40 strains were collected during 2009-2010 resistant to metronidazole, amoxicillin, ciprofloxacin, clarithromycin and tetracycline are 24(60%), 4(10%), to11(27%), 7(17%) and 2(5%) in 2010 respectively.

Conclusion: H. pylori resistances to nearly all of the studied antibiotics have been increased. Significant increase in resistance to metronidazole was the highlighted and concerning phenomena in current study, but it is still lower than some Asian countries. Regard to increasing resistant trend in H.pylori, an Iranian main health problem, it is needed to find appropriate alternative in antibiotics agents administration. Also it is suggested that antibiotic prescription and its offer in drug stores in Iran should be revised.

Keywords: antibiotic resistance, helicobacter pylori

In vitro and In vivo Effect of Ducrosia anethifolia on Candida albicans Compared with Ketoconazol

Nayereh Askari N, Eghtedardoost M*, Mostafavi A, Yaraee R
* maedoost@yahoo.com

ABSTRACT

Background and objective: Essential oil of Ducrosia anethifolia - one of native medicinal herbs in Iran-had been used to treat headache, and backache in traditional medicine. The antibacterial effects of some of its compounds have been studied previously. In this study in vitro and in vivo effect of the essence of Ducrosai anethifolia on Candida albicans as a fungal organism have been considered. The aims of this study are to evaluate in vitro antifungal activity of the essential oil on C. albicans growth and its synergic effect with ketoconazole, and also the effect in mouse candidiasis model.

Material and methods: In vitro antifungal activity of essential oil of D.anethifolia was investigated using standard broth microdilution method (from 4µg/ml to 15 mg/ml) against C.albicans (PTCC5027). The synergistic effect was also determined by combination of D.anethifolia oil with ketoconazole at various concentration. In vivo study carried out on mice model of candidal sepsis with 5-day administration of the essence or ketoconazol.

Results: MIC and MFC of the essence against C. albicans was determined as 1mg/ml and 2mg/ml respectively. Antifungal activity of ketoconazole has been increased 4 fold when administered in combination with essence of D. anethifolia. Kidney colony count of mice received D. anethifolia oil was the same as ketoconazol group and significantly less than control group ($P<0.05$).

Conclusion: Regarding to in vitro and in vivo antifungal activities of essential oil of D. anethifolia, especially in comparison with ketoconazole, it can be concluded that it has significant antifungal activity. Further studies needed in order to enlighten the exact mechanism of D. anthifolia antifungal activity.

Key words: Ducrosia anethifolia, Candida albicans, ketoconazole, antifungal, animal model.

Infection of Rhombomys opimus Due to Leishmania major at Zoonotic Cutaneous Leishmanianisis Focus in Damghan District

Mohammadi Azni S*, Rassi Y, Oshaghi MA, Mohebali M, Abai MR, Hajjaran H
*sadegh_azni@yahoo.com

ABSTRACT

Background and objective: Zoonotic Cutaneous Leishmaniasis is one of the most important infectious diseases in the world an Iran.. Rodents are the reservoirs of this disease. Identification of reservoirs is very important for planning control program.

Material and methods: This survey was performed as an experimental and practical study in Damghan district. To determine the reservoir host of the disease rodents were captured using Sherman live traps and identified. Impression smears were prepared from the ears of each rodent, and stained by Giemsa. The ear exudates were inoculated in RPMI1640 culture. DNA of Giemsa-stained slides and RPMI culture was extracted and followed by Nested PCR technique to amplification kDNA of Leishmania species.

Results: During this study 10 rodents from species of Rhombomys opimus were captured and identified. 4 out of 10 R.opimus (40%) was infected with Leishmania major.

Conclusion: High density of R.opimus in rural area and high infection rate of them to L.major are the most important reasons to introduce of R.opimus the main reservoir host of disease. So control of rodents in a radius of 500 meter from houses in the infected village is necessary.

Keywords: Zoonotic Cutaneous Leishmanianisis, Rhombomys opimus, –Leishmania major, PCR, Damghan

The Fauna and Ecology of the Sand Flies (Diptera:Psychodidae) in Zahedan County, Sistan – Baluchistan Province, Southeastern Iran

Kassiri H*[1] , Javadian E[2]
***** Hamidka_2000@yahoo.com

ABSTRACT

Background and objective: Phlebotomine sand flies are the vectors of various types of leishmaniasis in Iran . In order to understand the faunal composition and ecology of sand flies , this survey was carried out in the urban and rural areas of Zahedan district , south – east of Iran.

Materials and methods: A total of 13 cities an villages were screened for sand flies . Collections were made in 1997 – 1998 . The specimens were collected by sticky paper traps coated with castor oil were rolled up and placed in the outdoor places . The specimens collected were preserved in 70 percent alcohol and slides were prepared in Puri's medium . The sand flies were identified according to standard method .

Results: In this study 6323 specimens were collected and identified . We found nine species of Phlebotomus and thirteen species of Sergentomyia . P . papatasi (23.5%) , S . tiberiadis (17.7%) , S . clydei (14.2%) , S . sintoni (12.9%) , P . sergenti (9.1%) , P . alexandri (7.9%) and P . kazeruni (6.7%) were the predominant Phlebotomine species in our investigation . During the collection of sand flies , the species of , P . bergeroti , P . halepensis , P . keshishiani , P . major , S . africana , S . baghdadis , S . clydei , S . dentata , S . dreyfussi , S . grekovi , S . hodgsoni , S . iranica and S . sumbarica were collected for the first time from this county.

Conclusion: In this study P . papatasi had highest density in Zahedan district . High density of P . papatasi and P . sergenti in this district could indicate risk of spread cutaneous Leishmaniasis .

Keywords: Sand fly , Fauna , Ecology , Leishmaniasis , Iran.