

Biofilm Formation and Frequency of Fimbrial Genes among *Escherichia coli* Strains Isolated from Patients with Urinary Tract Infection in Zahedan during 2017

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Abstract

Background and objective: Biofilm formation is a major determinant in the development of urinary tract infections (UTIs) by uropathogenic *Escherichia coli* (UPEC). Biofilm formation requires a set of genes facilitating the initial adhesion, maturation, production of the extracellular polymeric matrix and subsequent dispersal of bacteria. Several cell surface factors such as flagella, fimbriae, curli and exopolysaccharide production are involved in attachment of bacterial cells to the urinary tract and biofilm development. In this study we showed the prevalence of biofilm producing *E. coli* strains isolated from patients with UTI in Zahedan and also the presence of biofilm-associated genes was assessed.

Materials and methods: During 2017, a total of 112 *E. coli* isolates were collected from patients with UTI in a referral hospital in Zahedan and were identified using common biochemical and PCR confirmatory diagnostic tests. The ability of *E. coli* strains to form curli and cellulose and also biofilm was measured using qualitative Congo red agar and quantitative microtiter plate assays, respectively. The presence of *csgA*, *papC*, *fimH*, *sfaS* and *afaI* biofilm-associated genes was tested by PCR assay.

Results: A total of 85 strains (76%) were identified and confirmed as *E. coli* using PCR test. Amongst all, 58 strains (68%), produced curli and cellulose and were biofilm positive, in which, 7, 88 and 5% exhibited *rdar*, *bdar* and *pdar* morphotypes, respectively. Moreover, 52, 31 and 17% of strains were able to form strong, moderate and weak biofilm, respectively. The genes *csgA*, *papC*, *fimH*, *sfaS* and *afaI* were detected in 98, 79, 71, 14 and 10% of strains, respectively.

Conclusion: The results of the present study revealed the high prevalence of biofilm producing *E. coli* strains with biofilm associated genes among patients with UTI in Zahedan. The presence of such genes which are associated with bacterial attachment indicating the important role of curli and fimbria in biofilm formation during UTI infections which highlighted them as important targets for therapeutic purposes.

Key words: Uropathogenic *Escherichia coli*, biofilm, curli, cellulose, fimbrial genes

Phenotypic Comparison of Biofilm Formation among *Staphylococcus aureus* Strains Isolated from Patients in Tehran and Isfahan

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Abstract

Background and objective: *Staphylococcus aureus* is known as a major pathogen that causes a wide range of clinical infections, that is able to produce biofilm and cause new resistance patterns which results in failure of the treatment process. The aim of this study was to investigate the phenotypic comparison of biofilm formation among *S. aureus* strains isolated from patients in Tehran and Isfahan.

Material and methods: A total of 115 and 119 suspected *S. aureus* isolated from patients with various infections were collected from a referral hospital laboratory in Tehran and Isfahan, respectively, during 2017 and identified at the species level using polymerase chain reaction (PCR) test. The ability of the strains to form biofilm was tested using qualitative Congo red agar (CRA) and quantitative microtiter plate (MTP) assays.

Results: All 234 isolated strains were confirmed as *S. aureus* using specific primers for *nucA* gene. According to the result of CRA test, 12 and 8% of *S. aureus* strains isolated in Isfahan and Tehran, respectively, produced black colonies and were slime positive. Moreover, in MTP assay, 12 and 7% of strains in Isfahan and Tehran, respectively, were able to form strong biofilm. Totally, 97% of strains in both cities formed strong and moderate biofilm.

Conclusion: Based on the results of this study, it could be concluded that the quantitative MTP test has higher sensitivity and specificity than CRA for biofilm assessment. On the other hand, the high prevalence of biofilm producing strains in Tehran and Isfahan is a serious warning and threat to public health.

Keywords: *S. aureus*, biofilm, patients, microtiter plate, Congo red agar

Functional Groups and Antimicrobial Activity of *Ficus religiosa* Aqueous Extract on Pathogenic Bacteria “in vitro”

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Abstract

Background and objectives: Today, the use of herbal compounds to control and treat poisoning and infections bacterial has been considered. *Ficus religiosa* is a perennial plant that belongs to the Moraceae family. This study aimed to evaluate of the functional groups of bioactive compounds and antibacterial activity of *Ficus religiosa* aqueous extract on number of pathogenic bacteria “in vitro”.

Materials and methods: The extraction of *Ficus religiosa* leaf with maceration method was done for 48 hours. Using Kirby-Bauer and well agar methods, the inhibition zone diameter (IZD) bacterial growth was measured by aqueous extract of *Ficus religiosa* leaves. The minimum inhibitory concentration (MIC) and the minimum bacterial concentration (MBC) were determined using the micro-dilution broth (in 96-well) and pour plate method, respectively. The functional groups of bioactive compounds of *Ficus religiosa* aqueous extract was evaluated by Fourier transform infrared spectroscopy (FTIR).

Results: *Staphylococcus epidermidis* with a IZD of 13.1 and 16.3 mm in Kirby-Bauer and well diffusion agar methods had the highest susceptibility to *Ficus religiosa* leaf extract. The MIC was for *Salmonella typhimurium*, *Enterobacter aerogenes*, *Staphylococcus epidermidis* and *Listeria innocua* 128, 64, 32 and 32 respectively. The MBC of *Ficus religiosa* aqueous extract was for *Salmonella typhimurium*, *Enterobacter aerogenes*, *Staphylococcus epidermidis* and *Listeria innocua* >512, 512, 128 and 256 respectively. The main peaks of *Ficus religiosa* aqueous extract factor groups were observed in wave numbers of 3395.9, 2976.55, 2929.4, 1568.96, 1423.13, 1311.24, 1168.22, 1068.3, 861.75, 833.14, 771.85, 665.61 and 616.14 cm^{-1} .

Conclusion: The *Ficus religiosa* aqueous extract had antimicrobial activity on *Salmonella typhimurium*, *Enterobacter aerogenes*, *Staphylococcus epidermidis* and *Listeria innocua*. Therefore, the use of *Ficus religiosa* aqueous extract could be suggested in pharmaceutical industry.

Keywords: *Ficus religiosa*, Micro-dilution broth, Kirby-Bauer, Bioactive compounds.

Comparison Activity of *Myrtus communis* and *Descurainia sophia* on Bacteria and Fungal Isolated from Urinary Tract Infections

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Abstract

Background and objective: In many studies of medicinal plants used to treat bacterial infections. *Myrtus communis* is the member of the Myrtaceae family, and *Descurainia sophia* is the member of the Brassicaceae family. *Myrtus communis* and *Descurainia sophia* because of the different chemical compounds have antimicrobial activity. The purpose of this study was to compare the antimicrobial effects of *Myrtus communis* and *Descurainia sophia* extracts on bacterial and fungal of urinary tract infection.

Materials and methods: In this study ethanolic and methanolic extracts of the *Myrtus communis* and *Descurainia sophia* was prepared by maceration method. Antimicrobial effect of plants extracts on four strains of gram-positive including *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococcus faecalis*, *Bacillus cereus* and six gram-negative strains including *Escherichia coli*, *Serratia Marsysns*, *Shigella flexneri*, *Klebsiella pneumoniae*, *Salmonella typhimurium*, *Pseudomonas aeruginosa* and *Candida albicans* with well and disc diffusion assays were studied. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration by tube dilution method (MBC) were determined.

Results: The lowest MIC and MBC of *Myrtus communis* was against *Bacillus cereus* (0.97 mg/ml) and the maximum amount was against *Enterococcus faecalis* and *Shigella flexneri* (62.5 mg/ml).

Conclusion: The ethanol and methanol extracts of *Myrtus Communis* extracts had antimicrobial effects on the microorganisms but *Descurainia sophia* extracts had no antimicrobial effects. Most of the plant antimicrobial effect was on gram-positive bacteria and *Shigella flexneri* was the only gram-negative susceptible strain. *Candida albicans* Showed resistance to the effects of extracts.

Keywords: *Descurainia sophia*, *Myrtus communis*, urinary tract infection

The Evaluation of 16S rRNA, rpoB and hsp65 Genes in Accurate Identification of Nontuberculous Mycobacteria (NTM) Species

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Abstract

Background and objective: Nontuberculous mycobacteria (NTM) are a large family of acid-fast bacteria that are widely distributed in the environment. Although most NTM species are saprophytic, about 30% of these species are associated with human diseases. NTM and Mycobacterium tuberculosis complex have common microbiological characteristics, such as induce similar immune response and same clinical manifestations. However, disease caused by NTM is a diagnostic challenge for physicians, because it cannot readily be distinguished from tuberculosis on the basis of clinical history, tuberculin skin test results, radiological patterns, and initial laboratory reports. Therefore, accurate detection of NTM is necessary due to different therapeutic strategies and should be considered seriously.

Materials and methods: In this study, 120 water samples were collected for isolation of NTM species, and cultured in a Lowenstein-Jensen medium after decontamination and processing. Then, accuracy and sensitivity of the three conserved genes (16S rRNA, rpoB and hsp65), were evaluated in identification of NTM species isolated.

Result: In this study, 87 NTM colonies were isolated from water samples. The results of the sequence analysis of the three conserved genes showed that the hsp65 gene compared to other two genes has a high degree of accuracy and sensitivity.

Conclusion: Unlike hsp65 gene, partial sequence analysis of the 16S rRNA and rpoB genes does not seem to be sufficient for recognizing NTM species.

Keywords: NTM, Identification, 16S rRNA, hsp65, rpoB genes