

Biofilm Formation among *Staphylococcus epidermidis* Strains Isolated from Healthy People in Isfahan

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Abstract

Background and objective: *Staphylococcus epidermidis* is known as the normal flora residing in the human skin unlike *Staphylococcus aureus* lacks different virulence factors of pathogenesis, so biofilm formation should be considered as the unique pathogenic element in these bacteria. Prevalence of biofilm producing strains in healthy people could be a significant health challenge. The aim of this study was the isolation and phenotypic and genotypic analysis of biofilm formation among *S. epidermidis* strains isolated from healthy people samples in Isfahan, Iran during 2016.

Materials and methods: Sampling was carried out from 100 healthy people and all isolated strains were identified at the species level using specific primers and their ability to form biofilm was determined using combination of qualitative Congo-Red agar and quantitative microtiter plate assay. In addition the presence of *icaABCD*, *aap* and IS256 genes was detected by polymerase chain reaction (PCR).

Results: Using PCR, all 123 isolated strains were confirmed as *S. epidermidis* strains. The quantitative biofilm assay showed that 12.2% of strains were able to attach strongly to polystyrene microplates. On the other hand, 6.5% of *S. epidermidis* strains, respectively, were able to form black colonies on Congo-red agar plates and were slime positive. The presence of *icaA*, *icaB*, *icaD*, IS256 and *aap* genes was limited to 3.3, 3.3, 3.3, 0 and 52 % of strains.

Conclusion: The results of this study are indicated the spread of biofilm producing and antibiotics resistance *Staphylococcus epidermidis* strains among healthy people in Isfahan city. Due to the fact that *S. epidermidis* strains are usually considered as the contaminant strains in the clinical samples, precise diagnosis of these strains in the clinical samples can be a great help to the specialists in diagnosing clinical infections. Presence of *icaA* and *icaD* genes with high prevalence of *aap* gene among biofilm producing *S. epidermidis* strains suggests that *ica*, *aap* and biofilm forming ability occur jointly in specific *S. epidermidis* clones and spread preferentially in the hospital and community.

Keywords: *Staphylococcus epidermidis*, biofilm, *ica*, *aap*, IS256, Congo-red agar, Microtitre plate assay

Biofilm and Curli Formation among *Escherichia coli* Strains Isolated from Urinary Infections in Tehran. 2016

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Abstract

Background and objective: Bacterial biofilms are associated with a large number of persistent and chronic infections. Biofilm forming bacteria are particularly resistant to antibiotics and immune defenses, which makes it hard to treat and eradicate biofilm-associated infections. The aim of this study was to investigate the biofilm and curli formation among *Escherichia coli* strains isolated from urinary infections in Tehran during 2016.

Materials and methods: A total of 200 urine samples were collected from patients with urinary tract infections from in Tehran and *E. coli* isolates were identified using specific primers. The ability of *E. coli* strains to form biofilm, curli and cellulose was measured using qualitative Congo-Red agar and quantitative microtiter plate assays.

Results: Amongst all isolates, 124 strains (62%) were identified as *E. coli* and 16 (13%), 19 (15%) and 11 (9%) were able to form biofilm and harbored rdar, bdar and pdar morphotypes, respectively. On the other hand, 78 strains (63%) were not able to form biofilm. Moreover, using microtiter plate assay 15 and 39% of strains were able to form strong and low level biofilm, respectively.

Conclusion: Our findings illustrated the important role of curli and cellulose in attachment and biofilm formation among *E. coli* strains isolated from patients with UTI infections

Keywords: *Escherichia coli*, UTI, biofilm, curli, cellulose, Congo-red agar, Microtitre plate assay

Microbial Contamination of Tahini, Flavored Tahini and Halva in Yazd, Iran. 2017

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Abstract

Background and objectives: Tahini is obtained from sesame seeds. Hawala ardeh comes from the addition of roots of Chubak plant, citric acid, sugar and other additives to tahini. The flavored tahini is made from tahini by adding comes from the addition of cocoa or chocolate powder to the tahini. The high fat content of these products, low activity water content, and high levels of nutrients in these products provide a good environment for the growth of pathogenic microorganisms. Contamination of these products is sometimes unacceptable for human consumption and is often show to be a potential health risk. Hence, this investigation was done to assess the microbial quality of sesame products in Yazd province in 2017.

Materials and methods: This cross-sectional study was conducted on 51 tahini, 60 Halva ardeh and 16 flavored tahini. Presence of pathogenic microorganism such as Escherichia coli, Enterobacteriaceae, coliform, yeasts and molds were analyzed by standard methods.

Results: Results revealed that in 51 tahini samples no microbial contamination was detected. The contamination level of flavored tahini to Enterobacteriaceae was 12.5% (n= 2) and mold was 12.5% (n= 2). In halva ardeh, About 6.66% (n= 4), 1.67% (n= 1), 5% (n= 3) and 1.67 % (n=1) of total samples were contaminated higher than standard level for Escherichia coli, Enterobacteriaceae, mold and yeast respectively.

Conclusion: The contamination is mainly because of poor quality of water, public unhygienic conditions associated with washing of services, poor personal hygiene. Results show that temperature changes, the moisture content that the product is exposed during the period of production, storage, distribution and consumption, health education of the staff, regular controlling and promotion of health standards may reduce contaminants of these products.

Keywords: *Halva, Tahini, Microbiological quality standards, contamination.*

Polymerase Chain Reaction (PCR) Assay for Molecular Detection of *Haemophilus Influenzae* Bacterium in Clinical Sample of Sinusitis

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Abstract

Background and Objectives: *Haemophilus influenza* is a human-restricted Gram-negative bacterium that is part of the normal nasopharyngeal flora of most humans. *H. influenzae* can cause diseases such as pneumonia, meningitis, bacteraemia, sinusitis and acute otitis media. Nonculture methods like PCR have become available during the last two decades, providing early and accurate diagnosis of bacterial diseases. The purpose of this study was to design a PCR assay for the rapid detection of *H. influenzae* bacterium.

Materials and methods: In this study the target gene was *P6* so the specific primers were designed for it. The PCR reaction was set up on the DNA genomic of *H. influenzae*. To create a positive control, the PCR product was cloned in the pTZ57R/T vector and was transformed into *E. coli* JM107. The sensitivity of this assay was tested by a serial dilution of the positive control. A total of 72 clinical samples collected from patients with sinusitis were analyzed by PCR.

Results: PCR results showed a band of the expected size 273 bp. Sensitivity results indicated that the limit of detection of the assay was 1 CFU/ml. No amplification was observed after PCR with any of the microorganisms tested. There was 19 (26%) positive numbers of *H. influenza* in the samples in this study.

Conclusions: The PCR method showed to be rapid, sensitive, highly specific, and cheaper than commercial methods. The PCR methods could be easily adopted by public laboratories of developing countries for diagnostic purposes.

Keywords: *Haemophilus influenzae*, rapid method, PCR

Prevalence of *tir* Gene in *E. coli* Strain Isolated from Cold Foods

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Abstract

Background and objective: *E. coli* resides in natural intestinal tract of human and warm blooded animals. This bacteria can be transferred (fecal, oral) into cold foods like salad, cold sandwich that are not heated and infect other people.

Materials and methods: In this research, using enriched and isolated culture media (e.g. nutrient broth and MacConkey agar and EMB agar) and performing biochemical experiments (e.g. sugars fermentation, movement and Andole and etc.) as well as molecular method PCR, it was cleared that only 25 samples out of collected 100 cold food samples (e.g. lettuce, sausage, cabbage and tomato) had *E. coli* bacteria and *tir* gene in this bacterium have *tir* gene suggesting that pathogenic bacteria *E. coli* is in 25 (25%) samples.

Results: From between collected 100 cold food samples (25 lettuce, 25 sausage, 25 cabbage and 25 tomato) cultured samples in the isolated culture media and Direct PCR experiment indicated that just 25 samples (25%) sausage have *tir* gene. Samples sausage have *tir* gene. In fact, the results of cultivation and without cultivation (directly) were similar in PCR and in PCR from samples from cultivation and without cultivation, 25% *tir* gene has been observed. **Conclusion:** This research indicated that the gene *tir* can be identified using direct PCR in cold foods used in cold sandwich and salads in a shorter time (24 h) compared to cultures in isolated media and biochemical experiments. The presence of *tir* in the collected samples indicates the pathogenicity of the bacterium *E. coli*. Therefore, earlier identification of this gene through molecular method PCR can result in distinguished pathogenic *Escherichia coli*.

Keywords: *E. coli*, molecular method PCR, *tir* gene, cold food

Isolation of Multiple Drug-Resistant Genes on Encoding Exfoliative Toxin B Plasmid by Multiplex-PCR in *Staphylococcus aureus* Skin Infection Samples

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Abstract

Background and objective: Exfoliative toxin is *Staphylococcus aureus* various factors that can caused colonization and virulence. The aim of this study was to investigate the presence of several antibiotic resistant genes on exfoliative B-encoding plasmids in *S. aureus* infected skin specimens.

Materials and methods: 100 wound samples collected from Tehran Hospitals. Bacteria were identified by standard biochemical and microbiological tests. Antibigram test was performed for *S. aureus* isolates. DNA was extracted of resistance *S. aureus*. Presence of antibiotic resistant *etb*, *aac*, and *msrA* genes were identified by Muliplex-PCR.

Results: From 100 samples, 60 samples were *S. aureus*. Resistance to erythromycin, gentamicin, tobramycin, ciprofloxacin and linzolid was 3 (5%), 1 (2%), 1 (2%), 1 (2%) and 0 (0%) respectively. Frequency of *msrA*, *aac* and *etb* genes were 29 (48.3%), 45 (75%) and 37 (61.7%) respectively. Frequency for simultaneously presence of the *etb* / *aac*, *etb* / *msrA*, *msrA* / *aac* and *msrA* / *aac* / *etb* gene were 28 (46.6%), 22 (36.6%), 22 (36.6%) and 18 (30%) strains.

Conclusion: In this study, *etb* gene was indicated in most strains. As also, investigation of virulence genes in *S. aureus* skin infection samples showed frequency of resistance pathogen genes were increased.

Keywords: *Staphylococcus aureus*, *Plasmid resistance genes*, *Exfoliative toxin*, *Skin infection*, *Multiplex-PCR*.

Comparison of Antifungal Effects of *Zataria multiflora* Boiss and Clove Essential Oils with Fluconazole and Nystatin against clinical isolates of *Candida albicans*

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Abstract

Background and objectives: Today, *Candida albicans*' drug resistance to existing antifungal drugs, and in the other hands some side effects that related to the using of synthetic antifungal effects in the living organisms are the reasons that consumers prefer natural antifungal. The aim of this study was to evaluate the antifungal effects of *Zataria multiflora* Boiss (avishan-e shirazi) and clove oils in comparison with fluconazole and nystatin against clinical isolates of *C. albicans* In vitro.

Materials and methods: Identification of clinical isolates of *C. albicans* were done by phenotypic and genotypic methods. To investigate the antifungal effect of each essential oils and drugs, the M27-A3 protocol recommended by the Clinical and Laboratory Standards Institute (CLSI) was used and Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) were determined.

Results: The MIC of clinical isolates for essential oils of *Zataria multiflora* Boiss and Clove were 0.003 and 0.47 µl / ml, respectively. The MIC was in the range of 2.37-9.5 IU / ml for nystatin and 3.12 - 12.5 µg / ml for fluconazole.

Conclusion: Our finding showed that the essential oils of *Zataria multiflora* Boiss and Clove have more anti-fungal activity against clinical isolates of *C. albicans* than fluconazole and nystatin. According to the results of this study, essential oils of *Zataria multiflora* Boiss and Clove are recommended for controlling the vulvovaginitis caused by *C. albicans*.

Key words: *Zataria multiflora* Boiss essential oil, Clove essential oil, *Candida albicans*, Fluconazole