

Biofilm Formation and Its Genes Expressions in *Staphylococcus epidermidis* Isolated from Urinary Tract Infections of Children in Isfahan

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Abstract

Aims: *Staphylococcus epidermidis* is an important bacterium, also one of the 40 species related to the *Staphylococcus* family. It can be found in the human normal body flora, commonly on the skin, and less commonly on mucosal flora. **Instrument and Methods:** In the cross-sectional study, we were isolated samples according to the laboratories standards, and *S. epidermidis* identification were collected for 1 year, 90 *S. epidermidis* from urinary tract infections of children were selected from educational hospitals in Isfahan, (Iran). In this way, we use the Kirby–Bauer method. *S. epidermidis* isolates were collected for determined biofilm producing method, with culturing in (Congo red agar) medium and microplate titration. **Results:** The results reveal that 45 methicillin resistance *S. epidermidis* isolates produce biofilm in different levels. The high resistance was for methicillin (50%), erythromycin (43.5%), ciprofloxacin (50.2%), and penicillin (46.9%). The lowest resistance was for linezolid (4%) and nitrofurantoin (5%). **Conclusions:** The results of our study show the high prevalence of antibiotic-resistant and biofilm producing of *S. epidermidis* strains, especially, in methicillin resistance *S. epidermidis* strains in the Isfahan hospitals, which could be a reservoir for antibiotic resistance genes.

Keywords: Biofilms, drug resistance, *Staphylococcus epidermidis*, urinary tract infections

INTRODUCTION

Urinary tract infections (UTIs) are a common problem in childhood and may be periodically benign which responding to simple antibiotic therapy or associated with a significant disruption in either the anatomy or function of a child's urinary system.^[1] This article will focus on (UTIs) affecting children, with an emphasis on those <2 years of age. Due to their more unique and complicated nature, neonatal (<28 days of age) UTIs will not be addressed as a specific issue. The principles discussed below, however, are applicable to that age group.^[1]

Staphylococcus epidermidis is an important bacterium, also one of the 40 species related to the *Staphylococcus* family. It can find in the normal flora of human, especially the skin,

and less in the mucosal flora. *S. epidermidis* is not pathogenic strain, but patients with weak immune systems can be at risk factor in developing infection.^[1,2]

These isolates infections are mostly hospital-acquired.^[3] *S. epidermidis* is one of the important concerns for patients with catheters or other surgical implants because of its ability to form biofilms that help it to grow on these devices. *S. epidermidis* is a famous and important microorganism, which is nonmotile,

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Gram-positive cocci, that can be classified into grape-like clusters. According to macroscopic view, it is white, raised, and colonies with 1–2 mm in diameter after incubation for 24 h, and also is not hemolytic on blood agar. A catalase-positive strain, coagulase negative, and facultative anaerobe can grow by aerobic breathing or by even fermentation.^[1-3] Some strains are aerobic.^[3] Biochemical tests demonstrate that *S. epidermidis* this also perform a weakly positive reaction to the nitrate reductase test. Urease manufacture is positive for *S. epidermidis*, oxidase is negative, *S. epidermidis* can use glucose as a sucrose, and lactose to produce acid and gas.^[4,5]

S. epidermidis does not have gelatinase enzyme and cannot hydrolyze gelatin. Sensitivity to novobiocin, create an important pattern to distinguish this strain from *Staphylococcus saprophyticus*, which is coagulase negative, but novobiocin-resistant.^[6]

Adherence to difference surface is the important step in generating and producing biofilm communities that is facilitated by the expression of different microbial surface components recognizing adhesive matrix molecules (MSCRAMMs) that can attach to several extracellular matrix factors such as elastin (ebpS), fibronectin A and B (fnbA and fnbB), laminin (eno), collagen (cna), fibrinogen (fib), and clumping factors (clfA and clfB).^[6] These genes can participate in joint signal sequence for attaching to the cell wall or surface. This protein in the bacterial matrix can coat medical devices and medical tools. Furthermore, can start protein producing from the inner section of the bacteria followed by attachment to the bacterial surface, which playing an important role for *S. epidermidis* pathogenesis and antibiotic resistance pattern.^[7]

In *S. epidermidis*, interactions with abiotic hydrophilic surface are controlled by polysaccharide intracellular adhesin which is encoded by the *Ica* operon, especially *icaABCD* that vintage is also involved in the combination of polysaccharide glucidic matrix, which can be affected by available anti-biofilm enzyme like herbal compounds. In strains that lack the *Ica* locus, biofilm formation is due to the presence of *aap* gene, which enables bacteria to bind to various matrix proteins. The insertion of transposon IS256 into the *Ica* locus results in a change of biofilm formation and resistance to aminoglycosides, which converts biofilm positive to biofilm negative bacteria.^[7] Conversion in the phenotype of biofilm cells is not wholly attributable. In our research, we decided to investigate effective genes that encoding MSCRAMMs, (using genotypic method in methicillin resistance *S. aureus*) during biofilm formation on Congo red agar (CRA) medium and polystyrene plates.^[8]

INSTRUMENT AND METHODS

Sample collection and identification

Samples and *S. epidermidis* identification was collected from May 2016 to March 2017 in Kashani Hospital of Isfahan in Iran, 90 *S. epidermidis* from UTIs of children (3–10 year) were selected from educational hospitals in Isfahan, Iran. All of the selected isolates were cultured in blood agar (Merck,

Germany), and then incubated at 37°C for 48 h. Afterward, suspicious colonies were examined with using techniques methods for recognize *Staphylococcus spp.* (microscopical morphology analysis, catalase, and coagulase production test). The 90 isolates were distinguished by conventional microbiological methods too, such as growth on mannitol salt agar (MSA) and deoxyribonuclease tests. *S. epidermidis* were known based on physical features of colony, gram staining, production of pigment in blood agar, hemolytic or the biochemical reactions similar to: catalysis activity, coagulase test (with plasma), and oxidase test, mannitol fermentation (MSA culture), urease activity, nitrate reduction, phosphates' test (Merck, Germany).^[9-12] *S. epidermidis* isolates were selected and antibiotic resistance pattern was performed by using the disk diffusion method by Mueller–Hinton agar.^[13]

S. epidermidis strains were tested with antibiotic disks such as amikacin (30 µg/disk), oxacillin (1 µg/disk), erythromycin (15 µg/disk), penicillin (5 µg/disk), tetracycline (30 µg/disk), tobramycin (10 µg/disk), gentamicin (120 µg/disk), rifampicin (2 µg/disk), sulfamethoxazole (1.20 µg/disk), ciprofloxacin (5 µg/disk), kanamycin (30 µg/disk), chloramphenicol (30 µg/disk), clindamycin (2 µg/disk), methicillin (5 µg/disk), nitrofurantoin (50 µg/disk), and linezolid (10 µg/disk) by disk diffusion method (Kirby–Bauer), (MAST, Merseyside, England), pursuant to Clinical and Laboratory Standards Institute 2011 method.^[13-15]

Biofilm organization (Congo red agar culture and microtiter plate)

The biofilm formation analysis was performed with tillage of the *S. epidermidis* strains, which detected from nosocomial infections on CRA plates (MAST, Merseyside, England), which used and characterized in the various study. The CRA cultures plate were incubated at 37°C in aerobic environment (24 h), and then, pursue by storage at room temperature for 48 h. The production of reddish, rough and black colonies, dry, crystalline consistency on CRA medium was considered as biofilm (slime) production. Nonslime producing (biofilm negative) strains produced pinkish red and smooth, colonies by a darkening point at its center. For complete this method, we used microtiter plate assay. In the way, we used polystyrene plate (MAST, Merseyside, England), 20 µL of isolates were append to polystyrene plate, and then incubated for 48 h at 37°C, then washing with phosphate buffered saline done, and safranin was used for staining strains in polystyrene plates, finally use ethanol to release biofilm producer isolates. We read absorbance with ELISA Reader (Biohit) (in 490 nm). The biofilm constructor isolates were chosen for biofilm gene (*icaABCD*) determination with molecular multiplex polymerase chain reaction methods.^[16]

DNA extraction and multiplex polymerase chain reaction amplification

One pure colony of the inquired *S. epidermidis* was cultivated in 1 mL (Tryptic Soy Broth) for 24 h at 37°C. Finally, the

bacterial DNA of *S. epidermidis* isolates were extracted with a QIAGEN plasmid (Mini Kit, Fermentas, Germany) as recommended in the kit.^[17]

S. epidermidis that was resistance to methicillin (by disc diffusions method) were selected, and biofilm producing of isolates were analyzed with phenotypic methods. All of the isolates were biofilm producer in different levels and all of the isolates selected for molecular amplification. ATCC 12228 was selected for nonbiofilm-forming *S. epidermidis*. Biofilm genes [Table 1] determined by specific primers, which are described in Dieter Vancraeynest and coworker study in Belgium, this gene listed in Table 1.

RESULTS

In the study, we were selected samples, and *S. epidermidis* identification was collected for 1 year, 90 *S. epidermidis* from urinary tract. In addition, we found that 45 *S. epidermidis* isolates of 90 samples (50%) were resistant to methicillin-resistant *S. epidermidis* (MRSE).

Forty-five methicillin resistance *S. epidermidis* isolates produce biofilm in different levels. The high resistance was for methicillin (50%), erythromycin (43.5%), ciprofloxacin (50.2%), and penicillin (46.9%). The lowest resistance was for linezolid (4%) and nitrofurantoin (5%). The reddish black formation of examined colonies with dry and rough colons with crystalline consistency on CRA medium was considered as slime production. Nonslime producing isolates manufactured pinkish red and smooth colonies with in a darkening at the center of colons. The phenotypic method shows that 57.2% of isolates were highly attached, 29.2% were selected as average biofilm producer, and 13.6% of isolates were low biofilm producer.

Study of this isolates, which selected from UTIs in children were selected from the hospital in Isfahan, show that the highest patients in his study were patients in 6–8-year-old. Frequencies of *S. epidermidis* isolates detected from different patients are shown in Table 2.

The frequency of genes that produce biofilm was *icaA* (32.6%), *icaB* (25.4%), *icaC* (72.3%), *icaD* (64.8%), 12.7% of patients did not have any history of UTI, 3.5% of patients were men, and 35% of patients were consumers. The *icaA* and *icaD* genes are encoding as important factors for intercellular adherence; it

could suppose that these genes can be such an important factors for the foundation of the different layer in cells with biofilm producing. The frequency of genes that produce biofilm show in Figure 1. Antibiotic resistance pattern of selected strains was determined with disk diffusion agar method are show in Table 3.

DISCUSSION

We showed the high prevalence of biofilm-producing *S. epidermidis* strains from May 2016 to March 2017 in Kashani Hospital of Isfahan isolated from hospitalized patients in Isfahan hospitals, Iran. Nearly, 90 *S. epidermidis* from UTIs were selected from Isfahan hospitals in Iran which most of them isolated from infections (UTI) of children in Isfahan.

It has been shown previously that catheters could be a risk factor for *Staphylococcal* infections.^[17] The phenotypic method show that 57.2% of isolates were highly attached, 29.2% were selected as average biofilm producer, and 13.6% of isolates were low biofilm producer that is higher than other research reports.^[16] On the other hand, in phenotypic methods, 45 methicillin resistance *S. epidermidis* isolates produce biofilm in different levels; this rate of biofilm production was higher than other studies.^[15] The results of this study are similar to Montanaro research in 2007.^[18,19]

The high resistances were for methicillin (50%), erythromycin (43.2%), ciprofloxacin (50.2%), and penicillin (46.9%). The lowest resistances were for linezolid (4%) and nitrofurantoin (5%) that is similar to other studies, among

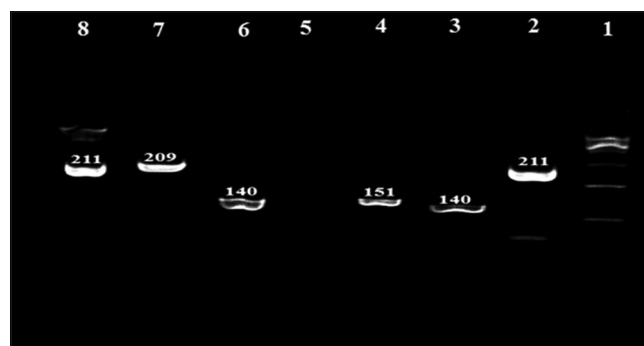


Figure 1: The frequency of genes that produce biofilm: *icaA*: 151 (bp)-*icaD*: 211 (bp)-*icaB*: 140 (bp)-*icaC*: 209 (bp)

Table 1: Primers of genes expression during biofilm formation			
Genes	Nucleotide sequence of primers (5' -3')	Amplicon size (bp)	Reference
<i>icaA</i> (intercellular adhesion gene)	5-GAGGTTAAAGCCAACGCACTC	151	[12]
	5-CCTGTAACCGACCAAGTTT		
<i>icaD</i> (intercellular adhesion gene)	5-ACCCAACGCTAAAATCATCG	211	[12]
	5-GCGAAAATGCCCATAGTTTC		
<i>icaB</i> (intercellular adhesion gene)	5-ATACCGGCGACTGGGTTTAT	140	[12]
	5-TTGCAAATCGTGGGTATGTGT		
<i>icaC</i> (intercellular adhesion gene)	5-CTTGGGTATTTGCACGCATT	209	[12]
	5-GCAATATCATGCCGACACCT		

Table 2: Frequency of *Staphylococcus epidermidis* isolates detected from different ages

The patient's age	Number and percentage of patients
1-3	15
3-6	12
6-8	43
8-10	20

Table 3: Antibiotic resistance pattern of *Staphylococcus epidermidis*

Antibiotics	Resistance, n (%)
Amikacin	14 (32.6)
Oxacillin	17 (52)
Erythromycin	19 (43.5)
Penicillin	21 (46.9)
Tetracycline	29 (65.2)
Tobramycin	20 (46.2)
Gentamicin	31 (70.6)
Rifampicin	15 (33.9)
Sulfamethoxazole	24 (54.2)
Ciprofloxacin	22 (50.2)
Kanamycin	15 (35)
Chloramphenicol	24 (53)
Clindamycin	28 (63.2)
Linezolid	2 (4)
Methicillin	22 (50)
Nitrofurantoin	3 (5)

biofilm and nonbiofilm, producing an *S. epidermidis* strain that is like to other studies.^[20-22]

Low rate of resistance to some antibiotics such as linezolid, clindamycin, tobramycin, tetracycline, and amikacin was surprising, yet this might be due to the no frequent use of such antibiotics for the treatment of infections caused by *S. epidermidis* strains in Isfahan, Iran.^[23]

The frequency of genes that produce biofilm was *icaA* (32.6%), *icaB* (25.4%), *icaC* (72.3%), *icaD* (64.8%); 12.7% of patients did not have any history of UTI. The *icaA* and *icaD* genes are encoding as necessary factors for intercellular adhesion.^[23,24]

In this research, 50% of *S. epidermidis* strains were resistant to methicillin and classified as MRSE strains; these values are higher than Piette and other reports.^[25] In conclusion, the results of our study show the high prevalence of antibiotic-resistant and biofilm producing of *S. epidermidis* strains, especially in methicillin resistance *S. epidermidis* strains in the Isfahan hospitals, which could be a reservoir for antibiotic resistance genes.^[26]

In our study, urinary isolates show a high percentage of biofilm production; however, the overall percentage of biofilm producing strains is much lower in our study than that in the one from Egypt in 2009.^[27] In conclusion, the results

of our study show the high prevalence of antibiotic-resistant and biofilm producing of *S. epidermidis* strains, especially in methicillin resistance *S. epidermidis* strains in the Isfahan hospitals, which could be a reservoir for antibiotic resistance genes.^[28] It is significant to note that both these genes were demonstrated in our biofilm-producing strains of *S. epidermidis*.

CONCLUSIONS

The results of our study show the high prevalence of antibiotic-resistant and biofilm producing of *S. epidermidis* strains, especially in methicillin resistance *S. epidermidis* strains. Further research is needed to contribute to the development of biomaterials and physical electrical barriers to impede bacterial colonization, and novel strategies for therapeutic intervention.

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Conflicts of interest

There are no conflicts of interest.

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