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High Prevalence of *icaABCD* Genes Responsible for Biofilm Formation in Clinical Isolates of *Staphylococcus aureus* From Hospitalized Children

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Background: The *icaABCD* genes encode a Polysaccharide Intercellular Adhesion (PIA), which is a tight structure protecting *Staphylococcus aureus* community against adverse environmental conditions. The *ica* dependent biofilm formation plays an important role in persistent infections in hospitalized patients.
Objectives: The aim of this study was to detect *icaADBC* genes encoding PIA among *S. aureus* isolates from children in Loghman Hospital of Tehran.
Materials and Methods: We collected 22 clinical specimens from hospitalized pediatrics and identified the isolates. Then, we detected *mecA* gene among Methicillin Resistant *S. aureus* (MRSA), *SCCmec* types and *icaABCD* gens by PCR assay and specific primers.
Results: Five isolates (22.7%) were methicillin resistant (MRSA) and *mecA* gene was detected among them. All the MRSA isolates harbored SCC*mec* type III. Prevalence of *icaA*, *icaB*, *icaC* and *icaD* in the isolates were 16 (73%), 14 (63.6%), 16 (73%) and 16 (73%), respectively. Moreover, all the MRSA strains were *icaADBC* positive.
Conclusions: Prevalence of *icaADBC* genes was relatively high among children and also all the four *ica* genes were detected among MRSA strains.

Keywords: Staphylococcus aureus; MRSA; Biofilms; Pediatrics

1. Background

S. aureus clinical isolates, especially methicillin resistant strains, are the causative agents of various clinical signs such as folliculitis, boils, impetigo and cellulitis, which are important in children (1, 2). *S. aureus* infections have been sharply increased during the recent years and associated with more mortality than other bacterial agents (3). Attachment and colonization is the first step for *Staphylococcus aureus* pathogenesis. Biofilm formation leads to bacterial resistance to higher concentrations of antimicrobial agents in addition to host immune responses (4). The self-produced polymeric matrices adhere to inert and living surfaces (5). Penetration of antibiotics reduces through *S. aureus* and *S. epidermidis* biofilms (6), although carbon and amino acids can be adsorbed by the biofilm layers (7). Some of special clonal complexes (e.g. clonal complex 8) are capable to adhere to different surfaces and produce a large amount of biofilm (8). The *icaADBC* genes, encoding PIA play important roles in biofilm formation among *S. aureus* and *S. epidermidis* isolates (9). Infections caused by isolates producing slime layer are difficult to treat. Many chronic infections due to *S. aureus*, especially through medical devices, are associated with biofilm formation (10, 11). Strong biofilm producer isolates are more virulent with severe post-surgical infections (12). The ica dependent biofilm formation develops by production of a polysaccharide inter cellular adhesion (PIA- PNSG/ poly- beta-1, 6-N-acetylglucosamine polymer) by the N-acetyl glucose aminyl transferase enzyme (13). Two *ica A and D* genes in the operon encode this enzyme. The other genes in this operon include *icaB* (polysaccharide deacetylase), *icaC* (transporter of PIA) and *icaR* (the regulatory gene). In Akiyama's study, all *S. aureus* strains tested in skin lesions of impetigo, atopic dermatitis and pemphigus were covered with glycocalyx and formed microcolonies (14). Systemic and intravenous Staphylococcal isolates have been shown to harbor *ica* genes as twice as the normal flora of healthy volunteers (13). Most reports have detected some of these genes.

2. Objectives

The aim of this study was to detect the *icaADBC* genes encoding PIA among clinical isolates of *S. aureus* from children in Loghman Hospital of Tehran.

3. Materials and Methods

We collected 22 *S. aureus* isolates and then identified them with coagulase, manitol fermentation, colony mor-

phology and DNase tests. Methicillin resistant isolates were identified in the phenotypic test by disk diffusion with oxacillin disk. Bacterial isolates were suspended in 200 μL of TE buffer and then lysostaphin was added (comprising 200 μL of TE buffer and 20 μL of lysostaphin [2 μg/mL, Sigma]). Genomic DNA of *S. aureus* isolates was isolated according to Straubinger method (15). The *mecA* gene was detected with specific primers indicated in Table 1 (16). PCR reaction mixture comprised of 9.5 μL distilled water (DW), 2 μL DNTPs (10 mM), 1.5 μL MgCl2 (50 mM), 1 μL of each primer, 3 μL 10X PCR buffer (200 mM), 2μL Taq polymerase (500 U) and 5 μL template DNA. The thermal profile included initial denaturation at 94°C for 5 min, followed by 30 cycles of 94°C (30 s), 55°C (30 s) and 72°C (30 s) and final extension of 72°C (4 min). Reaction mixture for *SCCmec* types was 94°C (1 min), 51°C (1 min), 72°C (1.5 min) and final extension of 72°C for 10 min. Moreover, thermal profile for *icaA* gene concluded with 94°C (5 min), followed by 30 cycle of 94°C (1 min), 52°C (30 s) and 72°C (1.5 min) with final extension of 72°C (10 min). The annealing temperature for *icaB*, *icaC* and *icaD* set as 55°C for 1 min (17), shown in Table 2. PCR products were electrophoresed in 1% gel agarose in 1X TBE buffer with staining of 1 μL of each loading buffer and gel red and then observed under UV emission. Pearson Chi-Square was used to data analysis. A $P < 0.05$ considered significant.

Table 1. Primers for the *mecA* and *SCCmec* Types Used in This Study

Primer	Sequence 5' → 3'	Size (bp)	Reference
mecA		147	(16)
	F: GTG AAG ATA TAC CAA GTG ATT		
	R: ATG CGC TATAGATTGAAA GGA		
SCCmecI		613	(16)
	F: GCTTTAAAGAGTGTCGTTACAGG		
	R: GTTCTCTCATAGTATGACGTCC		
SCCmecII		398	(16)
	F: CGTTGAAGATGATGAAGCG		
	R:CGAAATCAATGGTTAATGGACC		
SCCmecIII		280	(16)
	F: CCATATTGTGTACGATGCG		
	R: CCTTAGTTGTCGTAACAGATCG		
SCCmecIVa		776	(16)
	F: GCCTTATTCGAAGAAACCG		
	R: CTACTCTTCTGAAAAGCGTCG		
SCCmecV		325	(16)
	F: GAACATTGTTACTTAAATGAGCG		
	R: TGAAAGTTGTACCCTTGACACC		

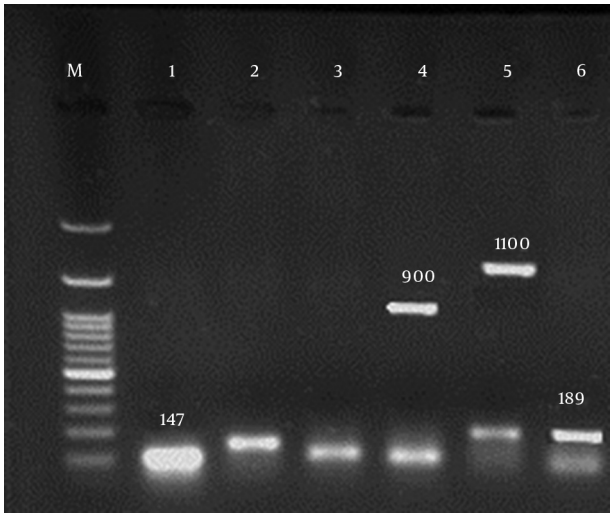
Table 2. Primers Sequences Used for Amplification of *icaADBC* Genes

Primer	Sequence 5' → 3'	Size (bp)	Reference
icaA		188	(17)
	F: ACACTTGCTGGCGCAGTCAA		
	R: TCTGGAACCAACATCCAACA		
icaB		900	(17)
	F: AGAATCGTGAAGTATAGAAAATT		
	R: TCTAATCTTTTTCATGGAATCCGT		
icaC		1100	(17)
	F:ATGGGACGGATTCCATGAAAAAGA		
	R: TAATAAGCATTAATGTTCAATT		
icaD		198	(17)
	F: ATGGTCAAGCCCAGACAGAG		
	R: AGTATTTTCAATGTTTAAAGCAA		

Table 3. Characteristics of MRSA Isolated From Hospitalized Children

MRSA	Clinical Origin	*mecA*	*SCCmec*	*ica* Genes	Gender
1	Trachea	+	III	ADBC	Male
2	Blood	+	III	ADBC	Male
3	Lesion	+	III	ADBC	Female
4	Trachea	+	III	ADBC	Male
5	Lesion	+	III	ADBC	Male

Figure 1. PCR Products Amplified in This Study



M: marker. columns 1, 3 and 4: *mecA gene*. column 2: *icaA* with 188 bp. columns 5 and 6: *icaD* gene with 189 bp. columns 4 and 5: *icaB* and *icaC* with 900 and 1100 bps, respectively.

4. Results

Five (22.7%) isolates were resistant to oxacillin (1 μg), moreover all the isolates were susceptible to vancomycin (2 μg) and linezolid (30 μg) disks. The *mecA* gene was detected in all five isolates (22.7%) with 147 bp size. All the MRSA isolates harbored *SCCmec* type III.

Furthermore, the prevalence of *icaA*, *B*, *C* and *D* in the isolates were 16 (73%), 14 (63.7%), 16 (73%) and 16 (73%), respectively as depicted in Figure 1. Interestingly, all MRSA isolates harbored all of *icaADBC* genes, suggesting that MRSA isolates may be more capable of PIA synthesis and biofilm formation (Table 3). However, there was no significant difference between MRSA and MSSA strains for the presence of *icaADBC* operon.

5. Discussion

All the studied isolates were susceptible to vancomycin and linezolid; although these drugs are the last choices for treatment of *S. aureus* infections, resistance to vancomycin has been sporadically reported from some areas of the world, similar to Iran (18, 19). Moreover, resistance to linezolid was detected in 19 of 20 isolates studied by Armin et al. (20). In this study, all MRSA strains harbored *SCCmec* type III. In the study of Japoni et al. (21) from south of Iran, *SCCmec* type III was the predominant type. MRSA strains harbor several virulence factors that develop more clinical signs (17, 22). Also the study by Imani Fouladi et al. (22), 75% of *Staphylococcus aureus* isolates with the SEB gene were Methicillin resistant and 15% were MSSA. In the study of Rahimi et al. (23), all the isolates were susceptible to vancomycin and most were susceptible to SXT. MRSA isolates harbored all *icaADBC* genes, suggesting that these isolates are strong biofilm producers and considered to cause chronic and persistent infections (24). Polymeric Intercellular Adhesion (PIA) plays an important role in attachment of bacteria to each other and to accumulate with multilayered biofilm. Catheter and blood stream Staphylococcal infections play an important role in biofilms (25, 26). We confirmed no significant difference between MSSA and MRSA isolates of *S. aureus* regarding the presence of *icaADBC* genes, similar to survey Atshan et al. (27), in which *icaADBC* genes were compared between MRSA and MSSA. Furthermore, we previously observed that most isolates belonged to accessory gene regulator (agr) group I (28), but the relationship of agr groups and *icaADBC* expression needs more studies. Several studies indicated the role of *icaA* and *icaD* genes in biofilm production and several reported that all of isolates were *icaA* positive (29). In the study by Hou et al. (30), among 55.56% of isolates that produced biofilm phenotypically, 11.11% had *icaA* gene, but other genes were not investigated. In this study, methicillin resistant isolates harbored higher rate of *icaADBC* genes, similar to studies conducted by Khan et al. (31) and O'neill et al. (32). However, Smith et al. (33) detected no significant correlation between susceptibility to methicillin and biofilm formation. Variations in the presence of *icaADBC* genes from studies might be due to epidemiological varieties and periods that these isolates have been collected.

Most of previous studies focused on the *icaAD* genes that encode PIA; likewise, these studies have not determined whether MRSA strains could produce PIA significantly more than MSSA isolates. For instance, Nasr et al. (34) detected *icaAD* genes in 32% of blood and catheter isolates. In the other study, 36 of 46 Staphylococcal isolates harbored *icaA* and *icaD* genes; while Grinholc and coworkers did not detect *icaD,* but all strains were *icaA*

positive (35). Terki et al. (36) detected *icaAD* genes in 17 (38.5%) of 44 staphylococcal isolates from urinary tract. In the other study, biofilm formation in most isolates was PIA dependent (37). Smith et al. (33) depicted that isolates of *S. aureus* from infected skin lesions were significantly more capable of producing biofilms than those isolated from blood and other infected sites. In the study of Semczuk et al. (38), all the isolates forming biofilm phenotypically, harbored *icaAD* genes. Satorres and Alcaraz (13) suggested that the *ica* genes might be more prevalent in *Staphylococcus* strains isolated from hospitalized patients or staff, than healthy individuals or the community. The limitations of this study were loss of healthy individuals, environmental strains and low number of isolates. In conclusion, the prevalence of *icaADBC* genes was high in hospitalized children in center of Tehran. There was no significant difference between MRSA and MSSA isolates of *S. aureus* regarding the presence of *icaADBC* genes, although all methicillin resistant strains harbored all the *icaADBC* genes.

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Authors' Contributions

Abdolmajid Ghasemian followed the microbiological and molecular laboratory studies. Shahin Najar Peerayeh designed the thesis of research, Bita Bakhshi and Mohsen Mirzaee advised the research.

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