

The Comparison of *Staphylococcus aureus* Isolated From Blood and Wound Specimens for Genes Encoding Polysaccharide Intercellular Adhesion (PIA)

Abdolmajid Ghasemian¹; Shahin Najar-Peerayeh^{1*}; Bitā Bakhshi¹

¹Department of Bacteriology, Tarbiat Modares University, Tehran, IR Iran

*Corresponding author: Shahin Najar-Peerayeh, Department of Bacteriology, Tarbiat Modares University, Tehran, IR Iran. Tel: +98-92182883870, Fax: +98-2182884555, E-mail: najarp_s@modares.ac.ir

Received: November 6, 2014; Revised: November 28, 2014; Accepted: December 17, 2014

Background: The polysaccharide intercellular adhesion (PIA) produced by *Staphylococcus aureus* is effective in the protection of isolates from outer harsh conditions and progress of infection.

Objectives: The aim of this study was to compare the *icaABCD* genes encoding polysaccharide intercellular adhesion (PIA) between blood and wound isolates of *Staphylococcus aureus* (*S. aureus*) in Tehran.

Patients and Methods: Forty-eight clinical isolates (including 30 blood and 18 skin wounds) were collected from patients and were identified. Next, *mecA* gene, *SCCmec* types and *icaABCD* genes were detected among blood and wound isolates of *S. aureus* by PCR assay and specific primers.

Results: Nine (19%) out of 12 methicillin resistant *S. aureus* (MRSA) isolates harbored *SCCmec* type III and three (6.2%) isolates harbored *SCCmec* type V. Prevalence of *icaA*, *icaB*, *icaC* and *icaD* in blood isolates was twenty-one (70%), fourteen (48%), nineteen (64%) and eighteen (60%), respectively; while the prevalence in wound isolates was as nine (50%), seven (39%), six (34%) and twelve (67%), respectively.

Conclusions: These findings showed no significant difference regarding the presence of *icaADBC* genes between blood and wound isolates.

Keywords: Methicillin-Resistant *Staphylococcus aureus*; Biofilm

1. Background

Staphylococcus aureus (*S. aureus*), especially those with methicillin resistance, are versatile pathogens capable of causing various clinical symptoms; ranging from mild and self-limited, to severe infections culminating in fatal outcomes (1). Staphylococcal bacteremia, particularly infections with methicillin resistant *S. aureus* (MRSA) isolates, has sharply increased during the recent years and is more strongly associated with mortality than other bacterial agents (2). Attachment and colonization is the first step for *S. aureus* pathogenesis. Biofilm formation allows the bacteria to resist higher concentrations of antimicrobial agents, environmental conditions and the host immune responses (3). The self-produced polymeric matrices (PIA) attach to inert and living surfaces (4). Penetration of antibiotics becomes impaired through *S. aureus* and *S. epidermidis* biofilms (5), although carbon and amino acids can be adsorbed by the biofilm layers (6). Infections with the ability to produce a slime layer are difficult to treat (7). Many persistent and chronic infections due to *S. aureus*, especially by medical devices, are particularly associated with biofilm formation (8, 9). Strong biofilm-producing isolates are more virulent, and cause severe post-surgical infections (10). The *icaADBC* genes play an important role in biofilm formation among both

S. aureus and *S. epidermidis* isolates. Among *ica* genes, *icaA* encodes the enzyme responsible for PIA synthesis. This enzyme requires the product of *icaD* (*IcaD*) for full activity (11). Co-expression of *icaA* and *icaD* induces higher enzymatic activity (12). The other genes within the *ica* gene cluster are *icaB* (polysaccharide deacetylase), *icaC* (transporter of PIA) and *icaR* (the regulator gene). In Akiyama's study, all *S. aureus* cells isolated from skin wounds of impetigo, atopic dermatitis and pemphigus produced glycocalyx and formed microcolonies (13). Most strains of *S. aureus* contain the all four genes of *ica* operon, although some reports have detected only some of these genes (7).

2. Objectives

The objective of this study was to detect *icaADBC* genes and to compare them between blood and wound isolates of *S. aureus*.

3. Patients and Methods

3.1. Clinical isolates

Thirty blood and 18 wound isolates of *S. aureus* were collected from July 2012 to January 2013. The isolates were

Table 1. Specific Primers for Genes Amplified in This Study

Primer, Sequence: 3' 5'	Primer Size	Reference
mecA F:GTG AAG ATA TAC CAA GTG ATT R: ATG CGC TATAGATTGAAA GGA	147	(15)
SCCmecI F: GCTTTAAAGAGTGTCTTACAGG R: GTTCTCTCATAGTATGACGTCC	613	(15)
SCCmecII F: CGTTGAAGATGATGAAGCG R: CGAAATCAATGGTTAATGGACC	398	(15)
SCCmecIII F: CCATATTGTGTACGATGCC R: CCTTAGTTGTCGTAACAGATCG	280	(15)
SCCmecIV F: GCCTTATTCGAAGAAACCG R: CTA CTCTTCTGAAAAGCGTCG	776	(15)
SCCmecV F: GAACATTGTTACTTAAATGAGCG R: TGAAAGTTGTACCCTTGACACC	325	(15)
icaA F: AACTTGCTGGCGCAGTCAA R: TCTGGAACCAACATCCAACA	188	(16)
icaB F: AGAATCGTGAAGTATAGAAAATT R: TCTAATCTTTTCATGGAATCCGT	900	(16)
icaC F: ATGGGACGGATTCCATGAAAAAGA R: TAATAAGCATTAAATGTTCAATT	1100	(16)
icaD F: ATGGTCAAGCCCAGACAGAG R: AGTATTTTCAATGTTAAAGCAA	198	(16)

confirmed with coagulase, mannitol fermentation, colony morphology and DNase tests.

3.2. Antibiotic Susceptibility Test

An antibiotic susceptibility test was performed according to Clinical and Laboratory Standards Institute (CLSI) Guidelines, with the Kirby Bauer assay. Antibiotic disks comprised of amoxicillin (10 µg), gentamycin (10 µg), tetracycline (30 µg), trimethoprim-sulfamethoxazole (25 µg), erythromycin (15 µg), clindamycin (2 µg), oxacillin (1 µg), vancomycin (30 µg), ciprofloxacin (5 µg) and linezolid (30 µg), (MAST, UK).

3.3. Genomic DNA Extraction

Bacterial isolates were suspended in 200 µL of tris-EDTA buffer, followed by addition of lysostaphin (comprising of 200 µL of TE buffer and 20 µL of lysostaphin [2 µg/mL, Sigma]). Briefly, after incubation for one hour, two steps of boiling were done for 15 minutes, and then the microtubes were centrifuged. Genomic DNA of *S. aureus* isolates was isolated according to the Straubinger method (14).

3.4. DNA Amplification

The *mecA* gene was detected with specific primers indicated in Table 1 (15). The PCR reaction mixture comprised of 9.5 µL distilled water (DW), 2 µL deoxyribonucleotide triphosphates (DNTPs) (10 mM), 1.5 µL MgCl₂ (50 mM), 1 µL of each primer, 3 µL 10 × PCR buffer (200 mM), 2 µL Taq polymerase (500 U) and 5 µL template DNA. The thermal profile included initial denaturation at 94°C for five minutes, followed by 30 cycles at 94°C (30 seconds), 55°C (30 seconds) and 72°C (30 seconds) and final extension at 72°C (four minutes). The reaction mixture for *SCCmec* types was at 94°C (one minute), 51°C (one minute), 72°C (1.5 minute) and final extension at 72°C for 10 minutes. Moreover, thermal profile for *icaA* gene concluded at 94°C (five minutes), followed by 30 cycles at 94°C (one minute), 52°C (30 seconds) and 72°C (1.5 minute) with final extension at 72°C (10 minutes). The annealing temperature for *icaB*, *icaC* and *icaD* was set at 55°C for one minute (16). The DNA of the positive control isolate for the genes was kindly provided by Dr. Ghaznavi Rad. We also used the reaction mixture without template as the negative control.

Table 2. Characteristics of Methicillin Resistant *S. aureus* Strains Tested in This Study^a

MRSA	CS	<i>mecA</i>	<i>SCCmec</i>	<i>ica</i> Genes	Antibiotic Resistance
1	lesion	P	III	A	Amx, Cip, E, T, CD
2	lesion	P	V	AD	Amx
3	blood	P	III	ADBC	Amx, Cip, T, CD, GM
4	blood	P	III	AD	Amx, Cip, E, T, CD, GM
5	blood	P	III	ADBC	Amx, T, E
6	lesion	P	V	A	Amx, Cip, E, T, CD
7	lesion	P	III	ADBC	Amx, Cip, E, T, CD, GM
8	lesion	P	III	o	Amx, Cip, E, T, CD, GM
9	blood	P	V	ADBC	Amx, T, Cip
10	blood	P	III	o	Amx, Cip, E, T, CD, GM
11	blood	P	III	ADBC	Amx, Cip, E, T, CD, GM
12	blood	P	III	ADBC	Amx, Cip, E, T, SXT, CD, GM

^a Abbreviations: Amx, amoxicillin; CD, clindamycin; Cip, ciprofloxacin; CS, clinical specimen; E, erythromycin; GM, gentamicin; SXT, trimethoprim-sulfamethoxazole; T, tetracycline.

3.5. Electrophoresis of Products

The PCR products were electrophoresed on 1% gel agarose, and were observed with 1 μ L of each loading buffer and gel red stains under UV emission.

3.6. Data Analysis

Student's t-test was used for data analysis. A P value of less than 0.05 was considered as significant.

4. Results

4.1. Antibiotic Susceptibility Test

Among the total of 48 isolates, 39 (81.2%) were resistant to amoxicillin, although all of the clinical isolates were susceptible to linezolid and vancomycin. Resistance to tetracycline, gentamycin, trimethoprim-sulfamethoxazole, erythromycin, clindamycin, ciprofloxacin and oxacillin were 58.4%, 21%, 17%, 18%, 16%, 17% and 25%, respectively. The MRSA strains were significantly more resistant to the majority of antibiotics. Blood and wound isolates had no significant difference regarding resistance to antibiotics.

4.2. The *mecA* Gene and *SCCmec* Types

Twelve (25%) isolates harbored *mecA* gene with a 147 base pair (bp) size. The majority of MRSA isolates (nine isolates or 19% of total) harbored *SCCmec* type III, followed by type V (three isolates or 6.2%)(Table 2).

4.3. Prevalence of *icaA*, *icaB*, *icaC* and *icaD* Genes

The prevalence of *icaA*, *B*, *C*, *D* in blood isolates was 21 (70%), 14 (48%), 19 (64%) and 18 (60%), respectively; while in wound isolates prevalence of these isolates was nine

(50%), seven (39%), six (34%) and 12 (67%), respectively. The *icaA* and *icaC* were more frequent in blood isolates. Fourteen (29.1%) blood isolates harbored all four *icaADBC* genes of the operon. The *icaA* and *icaD* genes were more frequent in the wound isolates. Seven (39%) Wound isolates had all four *icaADBC* genes. Also six out of twelve (50%) MRSA isolates harbored all *icaADBC* genes. There was no relationship between resistance to antibiotics and presence of *icaADBC* genes. The MRSA isolates harbored a high number of *icaADBC* genes, suggesting that MRSA may be more capable of producing PIA and biofilms.

5. Discussion

All studied isolates were susceptible to vancomycin and linezolid, although these drugs are the last resort for use against *S. aureus*. Resistance to vancomycin has been reported from several parts of the world in sporadic conditions, similar to Iran (17, 18). In our study, MRSA isolates were resistant to more antibiotics when compared to MSSA strains, and this difference between the two groups was significant for several antibiotics. However, one MRSA isolate, with *SCCmec* type V was susceptible to all antibiotics used, except for amoxicillin. This strain was isolated from a wound culture of a woman. Moreover, this isolate harbored the *icaAD* genes. The majority of MRSA strains in this study harbored *SCCmec* type III, which is important in healthcare associated infections or HA-MRSA infections. Similarly, our previous studies and Japoni's survey from south of Iran, depicted that *SCCmec* type III was the predominant *SCCmec* type (19-21). In this study, one MRSA with *SCCmec* type III was resistant to all the antibiotics, except for vancomycin and linezolid. This strain was isolated from a blood culture of a woman, and harbored all *icaADBC* genes, suggesting an isolate with strong biofilm production and

resistance to the used antibiotics. Biofilm formation protects *S. aureus* strains against environmental factors, antibiotics and host responses, and is considered to cause chronic and persistent infections (22). Polymeric intercellular adhesion (PIA) plays an important role in attachment of *S. aureus* strains to each other and accumulation of a multi-layered biofilm. Catheter and blood-stream Staphylococcal infections play an important role in biofilm formation and persistent infections (23, 24). There was no significant difference between blood and wound isolates of *S. aureus* regarding the presence of *icaADBC* genes, however most previous studies have not compared *icaADBC* genes between these two clinical sources. Several studies have detected *icaA* and *icaD* genes with high prevalence among *S. aureus* isolates (25) and several have reported that all the isolates were *icaA* positive. However, Hou reported that, 55.56% of isolates produced biofilms phenotypically, yet 11.11% harbored the *icaA* gene (26), while the other genes were not investigated. This study showed that *icaADBC* genes are more frequent in MRSA isolates, similar to Mirzaee, Khan and O'Neill studies (27-29). However, Smith believed that there is no significant correlation between susceptibility to methicillin and biofilm formation (30). The variations in the presence of *icaADBC* genes from different studies might be due to the epidemiological varieties and time at which clinical isolates were collected.

Similar to this study, several previous surveys have not exhibited significant differences between MRSA and MSSA isolates or between blood and wound isolates, but have shown that *icaAD* genes play important roles in PIA synthesis (31-34). Similarly, in this study, *icaADC* genes were the predominant genes among blood and wound isolates. However, Smith depicted that isolates of *S. aureus* from infected skin wounds were significantly more capable of producing biofilms than those isolated from blood and other infected sites (30).

There was no significant difference between blood and wound isolates of *S. aureus* regarding presence of *icaADBC* genes. The *icaADBC* genes were more frequent among MRSA isolates; but no significant difference was observed between MRSA and MSSA strains.

Acknowledgements

The authors acknowledge the Loghman Hospital staff for providing the clinical isolates.

Authors' Contributions

Abdolmajid Ghasemian performed the laboratory work. Shahin Najar Peerayeh designed the work, and Bitak Bakhshi advised the work.

Funding/Support

This work was supported by grants from Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran.

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