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The Microbial Surface Components Recognizing Adhesive Matrix Molecules (MSCRAMMs) Genes among Clinical Isolates of *Staphylococcus aureus* from Hospitalized Children

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KEY WORDS

Staphylococcus aureus
Surface Proteins
Virulence
Pediatrics
MRSA

ABSTRACT

Background: Isolates of *Staphylococcus aureus* express a myriad of adhesive surface proteins that play important role in colonization of the bacteria on nasal and skin surfaces, beginning the process of pathogenesis. The aim of this study was to screen several of the Microbial Surface Components Recognizing Adhesive Matrix Molecules (MSCRAMMs) genes among the isolate of *S. aureus* from hospitalized children.

Methods: A total of 22 *S. aureus* isolates were collected from hospitalized children in Tehran from 2012 to 2013. Detection of the *mecA* and several adhesive surface proteins genes including *clfA, B* (encoding clumping factors A, B); *fnbA, B* (encoding fibronectin binding proteins A, B); *fib* (encoding fibrinogen binding protein); *eno* (encoding laminin binding protein); *cna* (encoding collagen binding protein); *ebps* (encoding elastin binding protein) and *bbp* (encoding bone sialo-protein binding protein), was performed by PCR.

Results: The *clfAB* genes were detected among all the isolates. The prevalence of *fnbA, fnbB, fib, eno, cna, ebps* and *bbp* was 63%, 6%, 50%, 59%, 82%, 63%, 9% and 0%, respectively.

Conclusion: The high prevalence of these genes is important for future plans in vaccine designation. MRSA and MSSA isolates similarly can produce adhesive surface proteins for colonization.

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ARTICLE INFO

Received 18 Aug 2014;

Accepted 27 Sep 2014;

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Introduction

Staphylococcus aureus is a ubiquitous bacterial human pathogen in both community- and nosocomial sources, although referred as a human normal flora as well (1). These isolates asymptotically colonize on surfaces of healthy individuals, and thus the carriers can spread these infectious pathogens. *S. aureus* co-infections

with viral influenza cause deaths among children (2). Especially for Community-associated methicillin-resistant *S. aureus* (CA-MRSA) strains play important role in children infections via producing the Panton Valentine Leukocidin (PVL) and some other toxins. This pathogen has also been associated with high morbidity and mortality in pediatric oncology patients detected in pulmonary nodules (3).

Furthermore, several “new” clinical syndromes, such as severe sepsis and Waterhouse–Friderichsen syndrome, can cause high mortality among children (4).

Infants born to mothers with staphylococcal colonization have been more likely to be colonized (5). *S. aureus* primarily inhabits in the moist squamous epithelium in the anterior nares of colonized individuals (6), although the bacterium is capable of colonizing in a number of different anatomical sites including the nasopharynx via a numerous surface-attached (adhesive) and secreted proteins. Thus, colonized individuals may be at risk of endogenous infections with *S. aureus* that has entered in the sterile sites of the body through several routes, such as wounds and indwelling medical devices (7).

Microbial surface components recognizing adhesive matrix molecules (MSCRAMMs) on *S. aureus* surface, mediate staphylococcal adherence to components of the extracellular matrix of the host (8). These components are attached covalently to peptidoglycan by sortase enzymes (9). Furthermore, these components participate in biofilm formation, in addition to the *ica* operon that produces the polysaccharide intercellular adhesion [PIA] (10). Clumping factor A (ClfA) is the major staphylococcal fibrinogen (Fg) binding protein and is responsible for the observed clumping of *S. aureus* in blood plasma, culminating in arthritis and endocarditis (11). MSCRAMMs play a key role in initiation of endovascular, bone and joint and prosthetic-device infections (12). These structures can bind to molecules such as collagen (mostly via Cna), fibronectin (via FnbAB), and fibrinogen (with ClfAB and Fib) and thus evade immune system, and then can develop infections (13, 14). The aim of this study was to screen the MSCRAMMs genes among the isolates of *S. aureus* from hospitalized children.

Material and Methods

Bacterial isolates

A total of 22 *S. aureus* clinical isolates were collected from center of Tehran, Iran and also from different systemic infectious sites of hospitalized children, from July 2012 to January 2013. The isolates were identified with biochemical tests, such as mannitol fermentation on Mannitol Salt Agar (MSA) medium, slide and tube coagulase tests, DNase production, and colony morphology on blood agar medium.

The study was approved by Ethics Committee of the university.

Clindamycin inducible resistance

The Double disk diffusion (D-test) was performed in Muller Hinton Agar medium using clindamycin (2ug) and erythromycin (15ug) according to Clinical and Laboratory Standards Institute (CLSI) guidelines.

Detection of MRSA strains

Phenotypic detection of MRSA strains was conducted with oxacillin (1μg) (15) in the antibiotic susceptibility test. Moreover, PCR assay was performed to detect *mecA* gene with specific primers (Table 1).

Extraction of genomic DNA

One colony of each bacterial isolate was suspended in 200 μl of TE buffer, and then the enzyme lysostaphin was added (totally 200μl of TE buffer and 20μl of lysostaphin [2μg/ml, Sigma]). Genomic DNA of each *S. aureus* isolate was isolated according to Straubinger method (16).

PCR reaction

The purpose of using simplex and multiplex PCR was to determine *mecA* gene in MRSA and

Table 1
Sequences and sizes of primers used in this study

| Primer | Sequence 5' to 3' | Product size |
|-------------|--|--------------|
| <i>mecA</i> | F:5-GTG AAG ATA TAC CAA GTG ATT-3 R:5-ATG CGC TATAGATTGAAA GGA-3 | 147 |
| <i>clfA</i> | F: 5-ATTGGCGTGGCTTCAGTGCT-3 R: 5-CGTTTCTTCCGTAGTTGCATTTG-3 | 288 |
| <i>clfB</i> | F: 5-ACATCAGTAATAGTAGGGGCAAC-3 R: 5-TTCGCACTGTTTGTGTTTGCAC-3 | 204 |
| <i>fnbA</i> | F: 5-CATAAATTGGGAGCAGCATCA-3 R: 5-ATCAGCAGCTGAATCCCAT-3 | 128 |
| <i>fnbB</i> | F: 5-GTAACAGCTAATGGTCGAATTGATACT-3 R: 5-CAAGTTCGATAGGAGTACTATGTTC-3 | 524 |
| <i>fib</i> | F: 5-CTACAACTACAATTGCGTCAACAG-3 R: 5-GCTCTTGTAAGACCATTTTCTTTCAC-3 | 405 |
| <i>cna</i> | F: 5-AAAGCGTTGCCTAGTGGAGA-3 R: 5-AGTGCCTTCCCAAACCTTTT-3 | 192 |
| <i>eno</i> | F: 5-ACGTGCAGCAGCTGACT-3 R: 5-CAACAGCATCTTCAGTACCTTC-3 | 301 |
| <i>ebps</i> | F: 5-CATCCAGAACCAATCGAAGAC-3 R: 5-AGTTACATCATCATGTTTATCTTTTG-3 | 188 |
| <i>bbp</i> | F: 5-AACTACATCTAGTACTCAACAACAG-3 R: 5-ATGTGCTTGAATAACACCATCATCT-3 | 574 |

the genes encoding adhesive surface proteins including *clfAB*, *fnbAB*, *fib*, *eno*, *cna*, *ebps* and *bbp*. The specific primers for these genes have been depicted in Table 1.

Reaction mixture for *mecA* gene: 9.5µl Distilled water (D.W), 1µl primer, 1.5µl MgCl₂ (50mM), 3µl 10x buffer, 2µl dNTPs (10mM), 2µl Taq polymerase (500U) and 5µl DNA template.

Reaction mixture for *clfAB*, *fnbAB* and *fib* multiplex: 9.5µl D.W, 1µl primer, 1.5µl MgCl₂, 3µl 10x buffer, 2.5µl dNTPs, 2µl Taq polymerase and 5µl DNA template.

Reaction mixture for *eno*, *cna*, *ebps* and *bbp* multiplex: 9.5µl D.W, 1µl each primer, 1.5µl MgCl₂, 3µl 10x buffer, 2.5µl dNTPs, 2µl Taq polymerase and 5µl DNA template.

Data Analysis

Pearson Chi-Square was used for data

analysis. A *P*-value less than 0.05 was considered as significant.

Results

Antibiotic susceptibility test

In the antibiotic susceptibility test, all the isolates were susceptible to vancomycin and linezolid. Five (22.7%) isolates were resistant to oxacillin and *mecA* gene was detected among them. Two methicillin susceptible *S. aureus* (MSSA) isolates showed inducible resistance to clindamycin. However, this phenomenon was not observed among MRSA strains.

Detection of genes encoding adhesive surface proteins

All the isolates (MRSA and MSSA) harbored

clfA,B genes. The prevalence of *fnbA*, *fnbB*, *fib*, *eno*, *cna*, *ebps* and *bbp* was as 63,6%, 50%, 59%, 82%, 63%, 9% and 0, respectively (Fig 1, 2). However, the frequency of these genes among MRSA isolates was 80%, 40%, 80%, 100%, 60%, 0% and 0%, respectively. There was no significant difference between MRSA and MSSA regarding prevalence of MSCRAMMs genes. Among blood isolates, the *clfAB*, *fnbA*, *fnbB*, *fib*, *eno*, *cna*, *ebps* and *bbp* were detected in 100% (n= 4), 50% (n= 2), 75% (n= 3), 100% (n= 4),

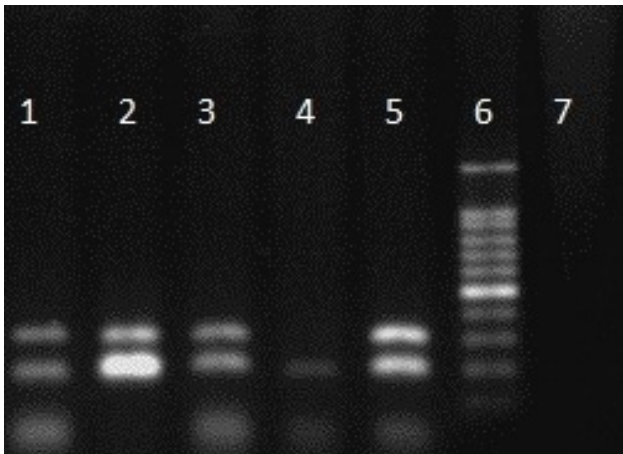


Fig. 1
Products of multiplex PCR for *eno*, *cna*, *ebps* and *bbp* genes. Columns 1- 3: *eno* and *cna* genes. Column 6: marker

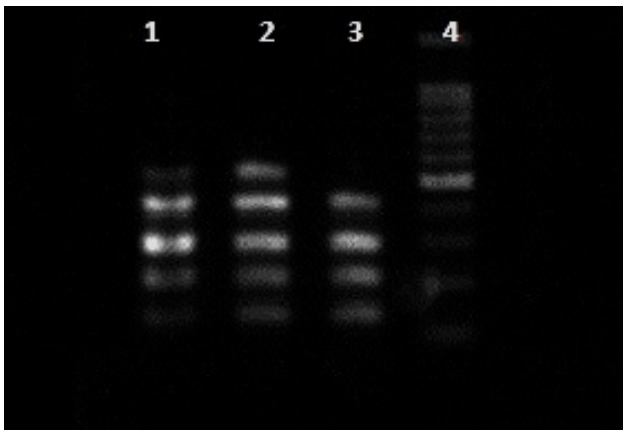


Fig. 2
Products of multiplex for *clfAB*, *fnbAB* and *fib* genes. Columns 1 and 2: *clfAB*, *fnbAB* and *fib* genes. Column 4: marker

75% (n= 3), 50% (n= 2), 25% (n= 1) and 0% (n= 0) of the isolates, respectively.

Discussion

In the present study, all the isolates were susceptible to vancomycin and linezolid. The glycopeptide antibiotics have remained the last resorts for treatment of MRSA infections (17). Morbidity and mortality due to MRSA have been frequently reported from many geographical areas (18). A previous study in Iran showed one vancomycin resistant (VRSA) isolate harboring the *vanA* gene (19). Two of our isolates had inducible clindamycin resistance. In our previous study, similarly this phenomenon was low (4%) (20).

In this study, five isolates were resistant to methicillin. We previously observed that all MRSA isolates exhibited SCCmec type III. MRSA strains, particularly those with SCCmec type III, represent serious human pathogens in Iran (21-23).

On the other hand, we previously determined that the majority of our isolates belonged to the accessory gene regulator (*agr*) group I (25), and there was no relationship between virulence genes and *agr* specific groups. The *agr* of *S.aureus* is a global regulator of the staphylococcal virulence genes, which include secreted virulence factors and surface proteins.

All the isolates examined in our study harbored *clfA,B* genes. Clumping factors play a critical role in attachment and colonization of *S. aureus*. Similar to our study, Klein et al. detected *clfB* in 91.8% of the isolates (26). There are no previous studies for *clfA,B* genes prevalence from Iran. Atshan et al. depicted that all strains harbored *clfA, B* genes (27). Moreover, Momtaz reported that nearly 20% of *S. aureus* isolates causing mastitis contain *clfA* gene (28). These studies suggest that *S.aureus* strains from different clinical sites may contain different frequencies of clumping factors, being essential for colonization. Furthermore, we observed that all the MRSA and MSSA strains, regardless of infection sites, harbored these two genes. In this

study, the frequency of *fnbA* and *fnbB* was 63.6% and 50%, respectively. We detected *fnbA* and *fnbB* genes in four (80%) and two (40%) MRSA isolates. The frequency was not significantly different from MSSA strains. The *fnbA* and *fnbB* genes were detected from two (50%) and three (75%) blood isolates. All isolates causing bloodstream infection harbored *clfAB* and *fnbAB* genes and produced strong biofilms (29). The fibrinogen binding protein gene (*fib*) was detected in all the isolates. Similar to our study, Bodén reported that the *fib* gene was present in all *S. aureus* strains (30). The prevalence of *eno* and *cna* genes was 82% and 63%, respectively, exhibiting the critical role of these genes during colonization of *S. aureus*. Duran et al. depicted that 78.4% of isolates harbored *cna* gene (31). In contrast to these, Arciola detected *cna* gene in 46% of isolates (32). The frequency of the *ebps* gene was 9%, however none of the isolates harbored *bbp* gene. These genes have been mostly detected among strains isolated from catheters and in addition from blood infections. In Paniagua and coworkers study, the most prevalent virulence genes were *clfA*, *clfB*, *cna*, *bbp*, *ebps* and *ica* (65.6 %, n = 21) among catheter related *S.aureus* isolates (33). By the phenotypic test, 26 (36.1%) strains were strong, 30 (41.6%) strains were moderate, and in 16 (22.3%) strains were weak biofilm producers (34). Tang though surveyed different sources, detected the *bbp* gene amplification only in one strain (35).

In the present study, there was no significant difference between MRSA and MSSA strains regarding biofilm production and frequency of MSCRAMMs genes. The source of infections can play a significant role in determination of pattern and expression of the predominant types of genes necessary for the attachment of *S. aureus*. To our knowledge, previous studies on the MSCRAMMs genes prevalence are scarce in Iran. Detection of these genes can help know the most prevalent exposing proteins and can culminate in developing new vaccine designs

according to Iranian isolates to prevent severe infections.

Conclusion

All the isolates (MRSA and MSSA) harbored *clfA,B* genes. Moreover, prevalence of *fnbA* and *eno* genes was high. The high prevalence of these genes is important for future plans in vaccine designation. MRSA and MSSA isolates similarly can produce adhesive surface proteins for colonization.

Acknowledgments

The authors acknowledge the efforts of Staff in Loghman Hospital of Tehran and their helps for collection of bacterial isolates. This study was supported by a grant from Tarbiat Modares University, Faculty of Medical Sciences, Tehran, Iran.

Conflict of interest

The authors declare that there is no conflict of interests.

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How to cite this article:

Ghasemian A, Najar Peerayeh SH, Bakhshi B. Mohsen Mirzaee The Microbial Surface Components Recognizing Adhesive Matrix Molecules (MSCRAMMs) Genes among Clinical Isolates of *Staphylococcus aureus* from Hospitalized Children. *Iran J Pathol.* 2015; 10(4): 258 - 264.