Comparison of the Prevalence of Microbial Surface Components Recognizing Adhesive Matrix Molecules (MSCRAMMs) among Staphylococcus aureus Isolates in a Burn Unit with Non-Burning Units

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Abstract
Background: Staphylococcus aureus (S. aureus) is one of the most important pathogens in burn infections colonized in the nose and increase the risk of infections.

Methods: Overall, 85 S. aureus isolates were isolated from clinical and nasal hospitalized patients and health care workers (HCWs) in a burn unit and non-burn units in Isfahan from June 2016 and September 2016. Genes encoding penicillin-binding protein 2a (mecA) and adhesive surface proteins, including fibronectin-binding proteins (fnbA, fnbB), fibrinogen binding protein (fibr), laminin-binding protein (eno), collagen binding protein (cna), elastin binding protein (ebpS), intracellular adhesion operon (icaA and icaD) were detected using PCR method.

Results: The rate of methicillin-resistant S. aureus (MRSA) among burn and non-burn isolates were 62% (18/29) and 25% (14/56), respectively. The most prevalent MSCRAMMs genes in burn units were eno (86%) and fibr (66%). The most common gene pattern in burn center was icaA+fibr+eno. The frequency of icaD, fibr and ebpS was higher in clinical samples than nasal samples. No relation was found between the MSCRAMMs genes in the burn unit and non-burn units.

Conclusion: The high prevalence of MRSA in burn center can be a new challenge for clinicians. The higher frequency of icaD, fibr and ebpS in clinical isolates than nasal isolates may reflect the important role of these genes in colonization and pathogenesis of S. aureus.

Keywords: Staphylococcus aureus; Methicillin-resistant Staphylococcus aureus (MRSA); Surface proteins; Proteins

Introduction

Staphylococcus aureus is an important pathogen with a variety of virulence factors that can cause life-threatening infections (1, 2). The bacteria is one of the most common causes of burn wound infections (3). The colonization of microorganisms in burn wounds may be the result of patient’s
endogenous flora or contact with contaminated environmental surfaces, the hands of healthcare workers (HCWs) and the air (4-6).

Nasal carriage of *S. aureus* plays a critical role in the development of *S. aureus* infection (7, 8). Biofilm production of *S. aureus* plays an important role in adherence and colonization of microorganisms on mammary epithelium cells and resistance to antibiotics or evasion from host immunological response. The intracellular adhesion (ica) operon is necessary for the control of biofilm production. Among ica locus, the icaA and icaD genes are more important than other genes (9, 10). The other virulence factors involved in adherence to the host tissue are microbial surface component recognizing adhesive matrix molecules (MSCRAMMs) which contains molecules called collagen-binding protein (Cna), elastin binding protein (EbpS), fibronectin-binding proteins (FnA and FnB), laminin-binding protein (Eno) and fibrinogen binding protein (Fib). Unfortunately, the emergence of multi-drug resistant strains has become a major public health concern worldwide (11). mecA acquisition converts methicillin-susceptible *S. aureus* (MSSA) strains into methicillin-resistant *S. aureus* (MRSA) that are resistant to different antibiotics (12-14).

We aimed to compare the frequency of genes encoding the MSCRAMMs among isolates of *S. aureus* from clinical and nasal samples in a burn unit with non-burning units at Isfahan, center of Iran.

**Methods**

Overall, 85 non-duplicate *S. aureus* isolates were collected from clinical samples and nasal swabs of hospitalized patients and HCWs in three hospitals, including a burn unit and two non-burn units in Isfahan, Iran from Jun 2016 and Sep 2016. Samples were obtained from Surgery, Intensive Care Units (ICUs), and Internal Medicine Wards.

The screening procedure for *S. aureus* nasal carriage was carried out by rotating a sterile swab soaked with saline in the anterior 1.5 cm of the nasal vestibule of both of the personnel and patient’s nares and inoculating into mannitol salt agar medium. Moreover, clinical samples were collected from hospitalized patients. Both clinical and nasal samples were transported to the laboratory of medical microbiology for identification. After incubation at 35 °C for 48 h, identification was performed based on colony morphology, Gram stain, catalase test, coagulase test, mannitol fermentation and DNase test (15). DNA templates for the PCR assay were extracted by the method (12). The presence mecA and MSCRAMMs genes were detected using specific primers as exhibited in Table 1. The PCR was performed in a 25 μl reaction mixture containing 1μl of each primer (10 pmol), 1X PCR buffer, MgCl2, 0.2 mM dNTP Mix, 5 μl of template DNA and 1.5U of Taq DNA polymerase. The Modified PCR conditions for MSCRAMMs and ica genes (fnbA, fnbB, fib, eno, cna, ebpS, icaA and icaD) were as follows: 25 cycles of denaturation at 94 °C for 1 min, annealing at 55 ° for 1 min and extension at 72 °C for 1 min (16, 17). Besides, PCR was performed for detection of mecA with the following amplification cycles: 30 cycles of denaturation (94 °C, 2 min), annealing (57 °C, 1 min) and extension (72 °C, 2 min).

**Statistical analysis**

The analysis was performed using SPSS™ software, version 21.0 (IBM Corp., USA). The results are presented as descriptive statistics in terms of relative frequency. Chi-square test was used to determine the significance of differences. A difference was considered statistically significant if the p-value was less than 0.05.

**Ethical approval**

This study was in accordance with the declaration of Helsinki and informed written consent was obtained from hospitalized patients and HCWs. The study protocol was approved by the Ethics Committee of Isfahan University of Medical Sciences (IR.MUI.REC.1394.3.951).
Table 1: Primers and product size for MSCRAMMs and biofilm genes

| Gene  | Primer sequence (5’-3’) | Product size (bp) | Reference |
|-------|-------------------------|------------------|-----------|
| cna   | F: GTCAAGCAGTTAATACACAGAC  
       | R: AATCATGTAATTGCTTGCAGTG | 423       | 16-17     |
| eno   | F: AAGTGGCAGACTGACT  
       | R: CAAAGGCTTCAGTTCCGATTC | 302       | 16-17     |
| fih   | F: GCACAGTTGCGTCAAAGAGCAG  
       | R: GCTCTTGTAAGACCATTTTTCAC | 404       | 16-17     |
| fnbA  | F: GTGAAGTTTTAGAAGGTGGAAAGATTAG  
       | R: GCTCTTGTAAGACCATTTTTCAC | 643       | 16-17     |
| fnbB  | F: GTTACAGCTAATGGTCGAATTGATACT  
       | R: CAAAGGCTTCAGTTCCGATTC | 524       | 16-17     |
| ebpS  | F: CATCCAGAACCAATCGAAGAC  
       | R: AGTTACATCATCATGTTTATCTTTG | 185       | 16-17     |
| icaA  | F: TGGCTGTATTAAGCGAAGAC  
       | R: CCGTCTTGTAAGACCATTTTTCAC | 669       | 16-17     |
| icaD  | F: ATGGTCAAGCCCAGACAGAG  
       | R: AGTATTTTCAATGTTTAAAAACAG | 198       | 16-17     |

Results

Of 85 S. aureus isolates 26 (31%) and 59 (69%) were clinical isolates and nasal isolates, respectively. Among 85 S. aureus isolates 32 (37.6%) isolates were MRSA and 53 (62.4%) were MSSA. Of 29 burn S. aureus isolates, 18 (62%) were MRSA while, of 56 non-burn S. aureus isolates, 14 (25%) were MRSA. The prevalence of MRSA was significantly higher in burn center than non-burn centers (P=0.0018).

In this study, the prevalence of icaA, icaD, cna, eno, ebpS, fib, fnbA and fnbB in MRSA isolates was as 46.8%, 46.8%, 25%, 84.3, 9.3%, 50%, 3.1% and 9.3%, respectively. In addition, the frequency of icaA, icaD, cna, eno, ebpS, fib, fnbB and fnbA in MSSA isolates was 49%, 33.9%, 35.8%, 62.2%, 43.3%, 58.4%, 9.4% and 3.7%, respectively. The prevalence of ebpS gene was significantly higher in MSSA than MRSA (P=0.0013). Comparison of the prevalence of virulence factors between clinical and nasal samples showed that the frequency of icaD (P=0.0176), fib (P=0.0095) and ebpS (P=0.0200) was significantly higher in clinical samples than nasal samples (Table 2). Furthermore, there was no significant difference between the frequency of MSCRAMMs genes in burn isolates and non-burn isolates. The comparison of prevalence of MSCRAMMs and ica genes in burn center with non-burn centers are shown in Table 2.

Table 2: The frequency of MSCRAMMs and biofilm genes among MRSA and MSSA isolates from burn unit and non-burn units in Isfahan, Iran

| Genotype | non-burn units n(%)=56 | burn unit n(%)=29 | P-value | Nasal isolates n(%)=59 | Clinical isolates n(%)=26 | P-value | Total n(%)=85 |
|----------|------------------------|------------------|---------|----------------------|--------------------------|---------|--------------|
| icaA     | 29(52)                 | 12(41)           | 0.4926  | 23(39)               | 18(69)                   | 0.0176  | 41(48)       |
| icaD     | 20(36)                 | 13(45)           | 0.4842  | 20(34)               | 13(50)                   | 0.2271  | 33(39)       |
| cna      | 19(34)                 | 8(28)            | 0.6285  | 17(29)               | 10(38)                   | 0.4508  | 27(32)       |
| eno      | 39(70)                 | 25(86)           | 0.1160  | 44(75)               | 20(77)                   | 1.0000  | 64(75)       |
| ebpS     | 21(37)                 | 5(17)            | 0.0815  | 12(20)               | 14(54)                   | 0.0042  | 26(31)       |
| fib      | 28(50)                 | 19(66)           | 0.2499  | 27(46)               | 20(77)                   | 0.0095  | 47(55)       |
| fnbA     | 4(7)                   | 1(3)             | 0.6569  | 3(5)                 | 2(8)                     | 0.6392  | 5(6)         |
| fnbB     | 5(9)                   | 1(3)             | 0.6590  | 5(8)                 | 1(4)                     | 0.4462  | 6(7)         |

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In this survey, high diversity in the coexistence of MSCRAMMs and *ica* genes was observed. The most prevalent coexistence profile was *icaA*+*icaD*+*fib*+*ebpS*+*cna*+*eno*, found in 8.2% (7/85) followed by *icaA*+*fib*+*eno* and *icaD*+*fib*+*eno* found in 7% (6/85) and 5.8% (5/85) of the isolates, respectively. The rate of other common pattern gene were *icaD*+*ebpS*+*eno* (4.7%), *fib*+*eno* (4.7%), *icaA*+*icaD*+*fib*+*eno* (3.5%) and *icaD*+*cna* (3.5%).

**Discussion**

In this study, the rate of MRSA in burn center was 62%, which is similar to reports conducted by Motallebi et al. (60.1%) (17) and Moghadam et al. (61.54%) (18) in Tehran but is higher than other reports in Iran (19, 20). Our results showed a significant difference in MRSA rate in burn center than non-burn centers in Isfahan. The higher prevalence of MRSA in burn centers than non-burn centers may indicate a potential outbreak of MRSA in the burn centers, which leads to treatment failure. According to our results, improvement of infection control programs and treatment guidelines in burn centers is recommended (21). A wide range of *S. aureus* strains carries the *ica* genes which leads to the production of biofilms in some of them. Therefore, the loss of *ica* cluster results in the reduction of strain capacity in biofilm formation (22). In this study, the presence of *icaA* and *icaD* was detected in 39% and 48% of *S. aureus* isolates, respectively. Various studies have shown a different rate of these genes which may indicate the difference in the source of strains in different geographical regions (17). A notable finding of the present study was a significantly higher percentage of the *icaD* gene in clinical isolates (69.2%) than nasal isolates (38.9%). Although the *icaD* gene is present in the nasal isolates, it may play a more important role in the development of the infection than *icaA* gene.

*Cna* adhesion is a virulence determinant which plays a role in the pathogenesis of septic arthritis, bone infection, endocarditis or bacteremia with bone or joint infection (23). In the present study, the rate of *cna* gene was 32% that is lower than the prevalence of other studies in the United Kingdom (52%), North America (43%), Sweden (57%), Turkey (78.4%) and higher than one study in Iran (17, 24-26). *fnbA* and *fnbB* are two adhesion factor genes that contribute to the invasion of bacteria (27). In the present study, the prevalence rate of *fnbA* was low (6%). In contrast, all *S. aureus* strains isolated from patients with Urinary Tract Infections (UTIs) were positive for *fnbA* (28). Interestingly, other studies in Iran and other countries reported a higher rate of this gene (9, 23, 27, 29). We observed that the frequency of the *fnbB* gene is also low (7%). *fnbB* was associated more with endocarditis than with osteomyelitis/arthritis (30). Another notable result in our study was higher prevalence *ebpS* (43.3%) in MSSA than MRSA (9.3%). These results are consistent with other studies that reported low prevalence *ebpS* in MRSA (17, 27, 29) and a high prevalence of this gene in MSSA isolates (31). The high frequency of *ebpS* in MSSA is an advantage for colonization and expansion of virulent clones. Furthermore, our study indicated that the higher proportion of *ebpS* and *fib* in clinical samples than nasal samples was statistically significant. Therefore, the strains carrying *ebpS*, *fib* and *icaD* genes have higher virulence potential and are more pathogenic than other strains.

In this survey, there was no significant difference between the frequency of MSCRAMMs genes in burn isolates and non-burn isolates but there were different coexistence patterns in the burn unit and non-burn units. *icaA*+*icaD*+*fib*+*ebpS*+*cna*+*eno* pattern was the most coexistence profile. Among 7 isolates with this profile, 6 isolates were collected from non-burn centers and one isolate from burn center. The most common profiles in burn center were *icaA*+*fib*+*eno* (5 isolates), *icaD*+*fib*+*eno* (3 isolates) and *fib*+*eno* (3 isolates). The high prevalence of *eno* (75%) in *S. aureus* isolates and the presence of *eno* and *fib* in the majority of coexistence profiles may reflect the critical role of these genes during
colonization of *S. aureus*, especially at the burn unit.

**Conclusion**

There was no significant difference in the prevalence of *ica* and MS-SCRAMMs in burn center with non-burn centers. However, the significantly higher frequency of *icaD*, *fib*, and *ebp* in clinical isolates than nasal isolates may reflect the important role of these genes in colonization and pathogenesis of *S. aureus*. This encourages the development of new strategies to prevent colonization of *S. aureus*. The high prevalence of MRSA in burn unit indicates the need to improve the control programs and treatment guidelines in burn units. Improvement of infection control programs and treatment guidelines in burn centers is recommended.

**Ethical considerations**

Ethical issues (including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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**Conflict of interest**

The authors declare that there is no conflict of interests.

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