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Original Article

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

Hossein SEDAGHAT [1], ***Tahmineh NARIMANI*** [2], ***Bahram NASR ESFAHANI*** [2], ***Sina MOBASHERIZADEH*** [3], ****Seyed Asghar HAVAEI*** [4,5]

1. *Department of Microbiology, Faculty of Medicine, Infectious Diseases Research Center, Kashan University of Medical Sciences, Kashan, Iran*
2. *Department of Microbiology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran*
3. *Nosocomial Infection Research Center, Isfahan University of Medical Sciences, Isfahan, Iran*
4. *Infectious Diseases and Tropical Medicine Research Center, Isfahan University of Medical Sciences, Isfahan, Iran*
5. *Department of Microbiology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran*

***Corresponding Author:** Email: havaei@med.mui.ac.ir

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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 *(mec*A) and adhesive surface proteins, including fibronectin-binding proteins (*fnbA, fnbB*), fibrinogen binding protein (*fib*), laminin-binding protein(*eno*), collagen binding protein (*cna*), elastin binding protein *(ebps),* intracellular adhesion operon (*ica*A and *ica*D) 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 *fib* (66%). The most common gene pattern in burn center was *ica*A+*fib*+*eno*. The frequency of *ica*D, *fib* and *ebp*S 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 *ica*D, *fib* and *ebp*S 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 *ica*A and *ica*D 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 (FnbA and FnbB), 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). *mec*A 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 *mec*A 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, ica*A and *ica*D) 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 *mec*A 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: GTCAAGCAGTTATTAACACCAGAC R: AATCAGTAATTGCACTTTGTCCACTG	423	16-17
eno	F: ACGTGCAGCAGCTGACT R: CAACAGCATCTTCAGTACCTTC	302	16-17
fib	F: CTACAACTACAATTGCGTCAACAG R: GCTCTTGTAAGACCATTTTCTTCAC	404	16-17
fnbA	F: GTGAAGTTTTAGAAGGTGGAAAGATTAG R: GCTCTTGTAAGACCATTTTTCTTCAC	643	16-17
fnbB	F: GTAACAGCTAATGGTCGAATTGATACT R: CAAGTTCGATAGGAGTACTATGTTC	524	16-17
ebpS	F: CATCCAGAACCAATCGAAGAC R: AGTTACATCATCATGTTTATCTTTTG	185	16-17
icaA	F: TGG CTG TAT TAA GCG AAG TC R: CCT CTG TCT GGG CTT GAC C	669	16-17
icaD	F: ATGGTCAAGCCCAGACAGAG R: AGTATTTTCAATGTTTAAAGCAA	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 *ica*A, *ica*D, *cna*, *eno*, *ebp*S, *fib*, *fnb*B and *fnb*A 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 *ica*A, *ica*D, *cna*, *eno*, *ebp*S, *fib*, *fnb*B and *fnb*A in MSSA isolates was 49%, 33.9%, 35.8%, 62.2%, 43.3%, 58.4%, 9.4% and 3.7%, respectively. The prevalence of *ebp*S 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 *ica*D (P=0.0176), *fib* (P=0.0095) and *ebp*S (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
icaD	29(52)	12(41)	0.4926	23(39)	18(69)	0.0176	41(48)
ica A	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)
*ebp*S	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)

In this survey, high diversity in the coexistence of MSCRAMMs and *ica* genes was observed. The most prevalent coexistence profile was *ica*A+*ica*D+*fib*+*ebp*S+*cna*+*eno*, found in 8.2% (7/85) followed by *ica*A+*fib*+*eno* and *ica*D+*fib*+*eno* found in 7% (6/85) and 5.8% (5/85) of the isolates, respectively. The rate of other common pattern gene are *ica*D+*ebp*S+*eno* (4.7%), *fib*+*eno* (4.7%), *ica*A+*ica*D+*fib*+*eno* (3.5%) and *ica*D+*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 *ica*A and *ica*D 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 *ica*D gene in clinical isolates (69.2%) than nasal isolates (38.9%). Although the *ica*D gene is present in the nasal isolates, it may play a more important role in the development of the infection than *ica*A 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).

*fnb*A and *fnb*B are two adhesion factor genes that contribute to the invasion of bacteria (27). In the present study, the prevalence rate of *fnb*A 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 *fnb*B gene is also low (7%). *fnb*B was associated more with endocarditis than with osteomyelitis/arthritis (30). Another notable result in our study was higher prevalence *ebp*S (43.3%) in MSSA than MRSA (9.3%). These results are consistent with other studies that reported low prevalence *ebp*S in MRSA (17, 27, 29) and a high prevalence of this gene in MSSA isolates (31). The high frequency of *ebp*S in MSSA is an advantage for colonization and expansion of virulent clones. Furthermore, our study indicated that the higher proportion of *ebp*S and *fib* in clinical samples than nasal samples was statistically significant. Therefore, the strains carrying *ebp*S, *fib* and *ica*D 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. *ica*A+*ica*D+*fib*+*ebp*S+*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 *ica*A+*fib*+*eno* (5 isolates), *ica*D+*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 MSCRAMMs in burn center with non-burn centers**.** However, the significantly higher frequency of *ica*D, *fib,* and *ebp*S 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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