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Spreading of genes encoding enterotoxins, haemolysins, adhesin and biofilm among methicillin resistant *Staphylococcus aureus* strains with staphylococcal cassette chromosome *mec* type IIIA isolated from burn patients

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Abstract

The emergence of antibiotic-resistant *Staphylococcus aureus* in particular methicillin-resistant *S. aureus* (MRSA) is an important concern in burn medical centers either in Iran or worldwide. A total of 128 *S. aureus* isolates were collected from wound infection of burn patients during June 2013 to June 2014. Multiplex-polymerase chain reaction (MPCR) assay was performed for the characterization of the staphylococcal cassette chromosome mec (SCCmec). Genes encoding virulence factors and biofilm were targeted by PCR. Of 128 *S. aureus* isolates, 77 (60.1%) isolates were MRSA. Fifty four (70.1%) isolates were identified as SCCmec type IIIA. The most frequently detected toxin genes among MRSA isolates with SCCmec type IIIA were *sea* (64.1%) and *hla* (51.8%). The rate of coexistence of *sea* with *hla* and *sea* with *hla* and *hlb* was 37% and 12.9%, respectively. The *sec, eta, tst, pvl, hla* and *hlb* genes were not detected in any of the MRSA isolates. The most prevalent genes encoding biofilm was *eno*, found in 61.1% of isolates, followed by *fib* and *icaA* found in 48.1% and 38.8% of the isolates, respectively. The rate of coexistence of *fib+eno+icaA+icaD* and *fib+eno* was 20.3% and 9.2%, respectively. The *ebps* gene was not detected in any of the isolates. In conclusion, our study indicated that the *sea, hla, fib* and *icaA* were most frequent genes encoding virulence factors among MRSA with SCCmec type IIIA isolated from burn wound infection. Moreover, the results of this study shows that the rate of coexistence of genes encoding different virulence factor were high.

**Key words:** MRSA, SCCmec type IIIA, MSCRAMMs, Toxin genes, Burn
1. Introduction

The emergence of antibiotic-resistant *Staphylococcus aureus* in particular methicillin-resistant *S. aureus* (MRSA) is an important concern in burn medical centers either in Iran or worldwide [1, 2]. Resistance to methicillin is due to the acquisition of *mecA* that is carried on a mobile resistance element, known as the staphylococcal cassette chromosome *mec* (SCC*mec*) [2]. Eleven main types of SCC*mec* differing in size and genetic composition have been described [3]. The larger SCC*mec* types (I-III) have been associated with healthcare-associated MRSA (HA-MRSA); whereas, the smaller SCC*mec* types (IV and V) usually have been associated with community-associated MRSA (CA-MRSA) [4].

The pathogenicity of *S. aureus* strains is related to various virulence factors including haemolysins (alpha, beta, gamma and delta), Panton-Valentine leukocidin (PVL), staphylococcal enterotoxins (SEs), toxic shock syndrome toxin 1 (TSST-1), exfoliative toxins (ETs), adhesion factors including microbial surface components recognizing adhesive matrix molecules (MSCRAMMs) such as fibronectin binding proteins (FnA and FnB), collagen binding protein (Cna), laminin binding protein (Eno), elastin binding protein (EbpS), fibrinogen binding protein (Fib) and biofilm associated protein (Bap) and ability for biofilm formation [5-10]. It has been found that the intracellular adhesion (*ica*) operon is essential for the control of biofilm production [8]. The *ica* locus, consisting of the gene *ica*ADBC, encodes the proteins mediating the synthesis of polysaccharide intercellular adhesin (PIA) molecule[9]. MRSA has emerged as important pathogen in Iran as in other countries, which presents serious challenges for burn patients [1, 2, 14]. The few reports of MRSA in Iran have suggested that MRSA with type III is common among burn patients but the spreading of genes encoding virulence factors and biofilm are unknown [2, 9]. Therefore, the aim of this study was to determine the frequencies of genes encoding virulence,
MSCRAMM and biofilm in a MRSA collection with SCC _mec_ type III obtained from the burn patients population of a teaching hospital in Tehran, Iran.

2. Materials and Methods

2.1. Bacterial isolates

A total of 128 _S. aureus_ isolates were collected from June 2013 to June 2014. These isolates were collected from wound infection of burn patients. Only one isolate per patient was included. Isolates were identified to species level using standard biochemical methods [9]. To confirm the identity of isolate as _S. aureus_ the _femA_ gene was amplified by a Polymerase Chain Reaction (PCR)-based method, using primers as described previously [6]. The _mecA_ gene was targeted by PCR using specific primers in order to identify isolate as MRSA [5, 11]. Isolates identified as MRSA were studied further.

2.2. SCCmec typing

Multiplex-PCR (MPCR) assay was performed to determine of SCCmec types, as described previously [3, 11]. The PCR conditions are as follows: MPCR-1 (for identifying of _mecA_ and _ccr_ genes), an initial denaturation step at 94°C for 2 min was followed by 30 cycles of amplification each, consisting of 94 °C for 2 min, annealing at 57 °C for 1 min and, extension at 72 °C for 2 min, ending with a final extension step at 72°C for 2 min [3]. The condition of M-PCR-2 that was used for identifying of _mec_ gene complex classes, A, B, and C2 was as M-PCR-1 except for annealing at 54 °C.

2.3. Detection of toxin genes

The oligonucleotide primers used in this study for detection of genes encoding haemolysins (_hla, hlb_), toxic shock syndrome toxin gene (_tst_), exfoliative toxin A gene (_eta_), staphylococcal enterotoxins (_sea, seb_ and _sec_) and Panton–Valentine leukocidin (_pvl_) among isolates with SCCmec type III are listed in Table 1 [5, 7, 8]. The PCR conditions consisted of
an initial denaturation step at 95°C for 5 min for each gene; followed by 30 cycles of 40 s at 95°C, 40 s at 54°C for annealing and 50 s at 72°C for hla and hlb, 60 s at 94°C, 120 s at 53°C, 120 s at 72°C for tst and eta, 120 s at 95°C, 120 s at 57°C, 60 s at 72°C for sea, seb-sec and 30 s at 94°C, 30 s at 55°C, 30 s at 72°C for pvl. A final extension step was performed at 72°C for 2 min.

2.4. Identification of MSCRAMMs and ica genes

The genes encoding adhesive surface proteins including fnbA, fnbB, fib, eno, cna, ebps, icaA and icaD were detected by PCR (table 2). The thermal cycling conditions used for amplification of the genes were a pre-denaturation step at 94°C for 5 min; 25 cycles of 1 min at 94°C, 1 min for annealing for each gene and 1 min at 72°C followed by 10 min final extension at 72°C.

3. Results

Of 128 S. aureus isolates, 77 (60.1%) isolates were MRSA. SCCmec typing of the 77 isolates produced 3 different SCCmec types. Fifty four (70.1%) isolates were identified as type IIIA, 2 (2.5%) as type II, 1 (1.2%) as type I and 20 (27.2%) isolates as nontypeable.

The prevalence of genes encoding virulence factors in isolates with SCCmec Type III is summarized in Table 3. The most frequently detected genes were sea (64.1%) and hla (51.8%). The rate of coexistence of sea with hla and sea with hla and hlb was 37% (20/54) and 12.9% (7/54), respectively. The sec, eta, tst, pvl, hla and hlb genes were not detected in any of the S. aureus isolates in this study.

The prevalence rate of MSCRAMMM and ica genes and the coexistence frequency of various genes among MRSA isolates with type IIIA are summarized in Table 4. The most prevalent gene was eno, found in 61.1% (33/54), followed by fib and icaA found in 48.1% (26/54) and 38.8% (21/54) of the isolates, respectively. The rate of coexistence of fib+eno+icaA+icaD and
fib+eno was 20.3% and 9.2%, respectively. The ebps gene was not detected in any of the isolates.

4. Discussion

MRSA is a pathogen of serious notice in Iran and a high prevalence rate of MRSA was previously reported in burn patients [6, 12, 13]. In this study the rate of MRSA isolates was 60.1%, which is higher than similar reports in Iran [6, 13], but lower than a report by Abbasi-Montazeri et al (80%) [12]. Several studies in worldwide have reported different prevalence rates for MRSA; for example 23% in Australia, 67% in Japan, 40% in South Pacific, 32% in USA, 26% in Europe, 67.4% in Iraq and 24.6% in Pakistan [14-16]. MRSA rates vary greatly among different countries, which may reflect differences in infection control policies and other factors. Although MRSA strains are not necessarily more virulent than methicillin-sensitive S. aureus strains, some MRSA strains contain factors or genetic backgrounds that may enhance their virulence or may enable them to cause particular clinical syndromes [17, 18].

In the current study, the 70.1% of MRSA strains were SCCmec type III and most of them (64.8%) carried the sea gene. A high prevalence rate of MRSA with SCCmec type III was previously reported in Tehran [5, 6]. In another study from burn patients, Namvar et al [2] reported that 47.5% of MRSA strains harbored SCCmec type III. The comparison of these two studies (the current study and Namvar et al, study), that have been done both in the same hospital, showed an increased prevalence of MRSA strains with SCCmec type III in recent years.

In the current study, 67.5% of all the MRSA isolates were positive for at least one of the virulence genes tested. This was lower than the data reported by Kim et al. [19], who had found that 85.5% of the MRSA isolates produced one or more toxins. Our study was in agreement with Degaim et al. [14], who reported that 72.9% isolates yielded positive results for sea [20]. In our
study 51.8% of isolates had *hla* gene and percentage of coexistence of *sea+hla* and *hla+hlb* genes were 37.5% and 1.8% respectively. Most of *S. aureus* isolated from human have usually a *hla*, because the human platelets and monocytes are more sensitive to the alpha toxin[21]. Kateete et al. [22] showed the frequency of *hla* gene was 100% [23]. Likewise, in a study from the United States the frequency of *hla* gene was reported as 100%. A study performed by Rusenova et al, showed that 56.2% of isolates have *hla* and *hlb* [24].

In this study, all the isolates were negative for the *pvl* gene. This may be due to the fact that the tested isolates carried the SCCmec types (I-III) as HA-MRSA while several studies have shown that the gene encoding PVL is the molecular marker for CA-MRSA which usually carries type IV SCCmec element [5, 7, 13]. In the current study, the fibrinogen binding protein gene (*fib*) was detected in 59.2% of the isolates and prevalence of *eno* gene was 61.1%, exhibiting the critical role of these genes during colonization of MRSA with SCC *mec* types III.

In the present study, the *cna* gene was present in 16.6% of MRSA strains with SCC *mec* types III. Our results were in agreement with other studies which reported a frequency of 22% [25] and 36% of *cna* positive strains[26]. This result was contradictory with another report which found that 63% of the tested strains were *cna* positive[27].

In the present study, more than 46.3% and 25.3% of the isolates harbored the *icaA* and *icaD* genes respectively, which is in accordance with the figures reported by Zmanta et al.[28]. However, the prevalence rates of the *icaA* and *icaD* genes vary greatly among different studies [29-32], which may reflect the difference in the origin or other factors of the isolates. A recent study by Paniagua et al reported that most prevalent virulence genes were *cna, ebps* and *ica* (65.6 %) among *S. aureus* isolates [33]. 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*.
Newly emerging MRSA clones appear to have acquired phenotypic traits that render them more virulent or able to colonize better, either via mobile genetic elements or adaptation of gene expression [34]. The difference in the prevalence of MRSA with SCCmec type III, virulence and adhesion genes might be due to differences in the geographical conditions of each country or region or part of the hospital where the specimens are collected.

In conclusion, our study indicated that the sea, hla, fib and icaA were most frequent genes encoding virulence factors among MRSA with SCCmec type IIIA isolated from burn wound infection. Moreover, the results of this study shows that the rate of coexistence of genes encoding different virulence factor were high.

Conflicts of interests
All contributing authors declare no conflicts of interests.

Acknowledgment
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References


[29] D. Campoccia, P. Speziale, S. Ravaiol, et al., The presence of both bone sialoprotein-binding protein gene and collagen adhesin gene as a typical virulence trait of the major


<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer Sequence (5’ to 3’)</th>
<th>Product Size, bp</th>
<th>Annealing temperature (°C)</th>
<th>Reference</th>
</tr>
</thead>
</table>
| sea  | F- GGT TAT CAA TGT GCG GGT GG  
       | R- CGG CAC TTT TTT CTC TTC GG | 102              | 57                        |           |
| seb  | F- GTA TGG TGG TGT AAC TGA GC  
       | R- CCA AAT AGT GAC GAG TTA GG | 164              | 57                        |           |
| sec  | F- AGA TGA AGT TGA TGT GTA TGG  
       | R- CAC ACT TTT AGA ATC AAC CG | 451              | 57                        |           |
| eta  | F- GCA GGT GTT GAT TTA GCA TT  
       | R- AGA TGT CCC TAT TTT TGC TG | 93               | 53                        | 5         |
| tst  | F- ACC CCT GCC TTA TCA TC   
       | R- TTT TCA GTA TTT GTA ACG CC | 326              | 53                        |           |
| hla  | F- CTT TCC AGC TTA CTG TAT CAG | 209              | 54                        |           |
| hlb  | F- GTG CAC TTA CTG ACA ATA GTG C  
       | R- GTT GAT GAG TAG CTA CCT TCA GT | 309          | 54                        |           |
| pvl  | F- ATCATAGGTAAAATGTCTGGACATGATCCA  
       | R- GCATCAASTGTATTGGATAGCAAAGC | 432              | 55                        |           |
### Table 2. Primers and annealing temperature for MSCRAMMs and biofilm genes

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer Sequence (5’ to 3’)</th>
<th>Product Size, bp</th>
<th>Annealing temperature (°C)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>cna</td>
<td>F: GTCAAGCAGTTATTAACACCAGAC&lt;br&gt;R: AATCAGTAATTTGCACTTTGTCACCCTG&lt;br&gt;F: ACGTGCAGCAGCTGACT</td>
<td>423</td>
<td>55</td>
<td></td>
</tr>
<tr>
<td>eno</td>
<td>R: CAACAGCATCTTCAGTACCTTC&lt;br&gt;F: CTACAACACTCAAATTGCGTCAACAG</td>
<td>302</td>
<td>55</td>
<td></td>
</tr>
<tr>
<td>fib</td>
<td>R: GCTCTTGTAAGACCATTTTCTTCAC&lt;br&gt;F: CTACAACACTCAAATTGCGTCAACAG</td>
<td>404</td>
<td>55</td>
<td></td>
</tr>
<tr>
<td>fnbA</td>
<td>F: GTGAAGTTTTTGAAGGTGGAAGATTAG&lt;br&gt;R: GCTCTTGTAAGACCATTTTCTTCAC</td>
<td>643</td>
<td>55</td>
<td>10</td>
</tr>
<tr>
<td>fnbB</td>
<td>F: GTAAACAGCTAATGTGCTGAAATTGATAC&lt;br&gt;R: CAAGTTGATAGGAGATTATGTAATCTTC</td>
<td>524</td>
<td>55</td>
<td></td>
</tr>
<tr>
<td>ebpS</td>
<td>F: CATCCAGAACCAATCGAAGAC&lt;br&gt;R: AGTTACATCATGTTTATCTTTTG</td>
<td>185</td>
<td>55</td>
<td></td>
</tr>
<tr>
<td>icaA</td>
<td>F: TGG CTG TAT TAA GCG AAG TC&lt;br&gt;R: CCT CTG TCT GGG CTT GAC C</td>
<td>669</td>
<td>55</td>
<td></td>
</tr>
<tr>
<td>icaD</td>
<td>F: ATGGCTAAGGCCCAGACAG&lt;br&gt;R: AGATTATACATGTTTAAAGCAA</td>
<td>198</td>
<td>55</td>
<td></td>
</tr>
</tbody>
</table>
**Table 3.** Distribution of toxin genes among MRSA isolates with SCCmec type IIIA

<table>
<thead>
<tr>
<th>Toxin Genes</th>
<th>N(%) of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>sea</td>
<td>7 (12.9)</td>
</tr>
<tr>
<td>seb</td>
<td>1 (1.8)</td>
</tr>
<tr>
<td>Sea+hla</td>
<td>20 (37)</td>
</tr>
<tr>
<td>Sea+hlb</td>
<td>1 (1.8)</td>
</tr>
<tr>
<td>hla+hlb</td>
<td>1 (1.8)</td>
</tr>
<tr>
<td>Sea+hla+hlb</td>
<td>7 (12.9)</td>
</tr>
</tbody>
</table>
Table 4. Distribution of adhesion and biofilm genes among MRSA isolates with SCCmec type IIIA

<table>
<thead>
<tr>
<th>Adhesion genes</th>
<th>N(%) of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>cna</td>
<td>1(1.8)</td>
</tr>
<tr>
<td>fib</td>
<td>1(1.8)</td>
</tr>
<tr>
<td>eno</td>
<td>2(3.7)</td>
</tr>
<tr>
<td>icaA</td>
<td>1(1.8)</td>
</tr>
<tr>
<td>fib+eno</td>
<td>5(9.2)</td>
</tr>
<tr>
<td>eno+icaA</td>
<td>2(3.7)</td>
</tr>
<tr>
<td>fib+icaA</td>
<td>1(1.8)</td>
</tr>
<tr>
<td>eno+icaD</td>
<td>1(1.8)</td>
</tr>
<tr>
<td>cna+fib+eno</td>
<td>2(3.7)</td>
</tr>
<tr>
<td>fib+eno+icaA</td>
<td>4(7.4)</td>
</tr>
<tr>
<td>fib+eno+icaA+icaD</td>
<td>11(20.3)</td>
</tr>
<tr>
<td>cna+fib+eno+icaA</td>
<td>1(1.8)</td>
</tr>
<tr>
<td>cna+fib+eno+fnbA+icaA</td>
<td>1(1.8)</td>
</tr>
<tr>
<td>cna+fib+eno+fnbB+icaA</td>
<td>1(1.8)</td>
</tr>
<tr>
<td>cna+fib+eno+fnbB+icaA+icaD</td>
<td>2(3.7)</td>
</tr>
<tr>
<td>cna+fib+eno+fnbA+icaA+icaD</td>
<td>1(1.8)</td>
</tr>
</tbody>
</table>
A total of 128 *S. aureus* isolates from burn wound infection were analyzed.

54 isolates were identified as MRSA with SCCmec type IIIA.

The most frequently detected toxin genes among were *sea* (64.1%) and *hla* (51.8%).

The *sec, eta, tst, pvl, hla* and *hlb* genes were not detected in any of the MRSA isolates.

The rate of coexistence of *fib+eno+icaA+icaD* and *fib+eno* was 20.3% and 9.2%, respectively.