# Accepted Manuscript

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

Mitra Motallebi, Fereshteh Jabalameli, Kheirollah Asadollahi, Morovat Taherikalani, Mohammad Emaneini

Please cite this article as: Motallebi M, Jabalameli F, Asadollahi K, Taherikalani M, Emaneini M, 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, *Microbial Pathogenesis* (2016), doi: 10.1016/j.micpath.2016.05.017.

# 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

**Mitra Motallebi[a], Fereshteh Jabalameli[a], Kheirollah Asadollahi[b], Morovat Taherikalani[c], Mohammad Emaneini[a*]**

[a] Department of Microbiology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran.

[b] Department of Social Medicine, Faculty of Medicine, Ilam University of Medical Sciences, Ilam, Iran.

[c] Department of Microbiology, School of Medicine, Lorestan University of Medical Sciences, Khoramabad, Iran.

* Corresponding author

E-mail: emaneini@gmail.com

**Address**

Department of Microbiology, School of Medicine, Tehran University of Medical Sciences

Building No. 6, 100 Poursina St., Keshavarz Blvd., Tehran 14167-53955, Iran

**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* (SCC*mec*).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 SCC*mec* type IIIA. The most frequently detected toxin genes among MRSA isolates with SCC*mec* 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% and12.9%, 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 SCC*mec* 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, SCC*mec* 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 (FnbA and FnbB), 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,

3

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. SCC*mec* typing

Multiplex-PCR (MPCR) assay was performed to determine of SCC*mec* types, as described previously [3, 11]. The PCR conditions are as follows: MPCR-1 (for identifying of *mecA* and *cc*r 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 SCC*mec* 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, 40s at 54°C for annealing and 50s at 72°C for *hla* and *hlb*, 60s at 94°C,120s at 53°C,120s at 72°C for *tst and eta*, 120s at 95°C,120s at57 °C,60s at72°C for *sea, seb- sec* and 30s at 94°C,30s  at 55°C,30s 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. SCC*mec* typing of the 77 isolates produced 3 different SCC*mec* 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 SCC*mec* 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 MSCRAMM 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

5

*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 SCC*mec* type III and most of them (64.8%) carried the *sea* gene. A high prevalence rate of MRSA with SCC*mec* 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 SCC*mec* 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 SCC*mec* 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 SCC*mec* 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 SCC*mec* 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 SCC*mec* 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 SCC*mec* 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**

**References**

[1] M.V. Rodrigues, C.M. Fortaleza, D.F. Riboli, et al., Molecular epidemiology of methicillin-resistant *Staphylococcus aureus* in a burn unit from Brazil, Burns. 39 (2013) 1242-1249.

[2] A.E. Namvar, M. Afshar, B. Asghari, et al., Characterisation of SCC*mec* elements in methicillin-resistant *Staphylococcus aureus* isolated from burn patients, Burns. 40 (2014) 708-712.

[3] T. Ito, K. Kuwahara-Arai, Y. Katayama, et al., Staphylococcal Cassette Chromosome *mec* (SCC*mec*) analysis of MRSA, Methods Mol Biol. 1085 (2014) 131-148.

[4] Y. Yancheng, C. Hang, Z. Renjie, et al., Application of the SCC*mec* element in the molecular typing of methicillin-resistant *Staphylococcus aureus*, Yi Chuan. 37(2015) 442-451.

[5] M. Emaneini, L. Jabalameli, H. Iman-Eini, et al., Multiple-locus variable number of tandem repeats fingerprinting (MLVF) and virulence factor analysis of methicillin resistant *Staphylococcus aureus* SCC*mec* type III. Pol J Microbiol. 60 (2011) 303-307.

[6] B. Fatholahzadeh, M. Emaneini, G. Gilbert, et al., Staphylococcal cassette chromosome *mec* (SCC*mec*) analysis and antimicrobial susceptibility patterns of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates in Tehran, Iran. Microb Drug Resist. 14 (2008) 217-220.

[7] S. Jarraud, C. Mougel, J. Thioulouse, et al., Relationships between Staphylococcus aureus genetic background, virulence factors, agr groups (alleles), and human disease. Infect. Immun. 70 (2002) 631-641.

[8] M. Mehrotra, G. Wang, W.M. Johnson, Multiplex PCR for detection of genes for *Staphylococcus aureus* enterotoxins, exfoliative toxins, toxic shock syndrome toxin 1, and methicillin resistance. J Clin Microbiol. 38 (2000) 1032-1035.

[9] P.R. Murray, American Society for M. Manual of clinical microbiology. Washington, D.C.: ASM Press; 1999.

[10] D. Vancraeynest, K. Hermans, F. Haesebrouck, Genotypic and phenotypic screening of high and low virulence *Staphylococcus aureus* isolates from rabbits for biofilm formation and MSCRAMMs. Vet Microbiol. 103 (2004) 241-247.

[11] S. Soroush, F. Jabalameli, M. Taherikalani, et al., Investigation of biofilm formation ability, antimicrobial resistance and the staphylococcal cassette chromosome *mec* patterns of methicillin resistant *Staphylococcus epidermidis* with different sequence types isolated from children. Microb. Pathog. 93 (2016) 126-130.

[12] E. Abbasi-Montazeri, A.D. Khosravi, M.M. Feizabadi, et al., The prevalence of methicillin resistant *Staphylococcus aureus* (MRSA) isolates with high-level mupirocin resistance from patients and personnel in a burn center. Burns. 39 (2013) 650-654.

[13] S.M. Hoseini Alfatemi, M. Motamedifar, N. Hadi, et al., Analysis of Virulence Genes Among Methicillin Resistant *Staphylococcus aureus* (MRSA) Strains. Jundishapur J.Microbiol. 7(2014) e10741.

[14] Z.D. Degaim, W.S. Shani, S.S. Hamim, Virulence factors of Methicillin Resistant *Staphylococcus aureus* (MRSA) isolated from burn patients. Int J Curr Microbiol App Sci. 4 (2015) 898-906.

[15] Z.A. Naqvi, K. Hashmi, S.A. Kharal, Methicillin resistant *Staphylococcus aureus* (MRSA) in burn patients. Pak Jour Pharmacol. 24 (2007) 7-11.

[16] K.S. Carvalho, E.M. Mamizuka, P.P. Gontijo Filho, Methicillin/Oxacillin-resistant *Staphylococcus aureus* as a hospital and public health threat in Brazil. Braz. j. infect. dis, 14 (2010) 71-76.

[17] R.J. Gordon, F.D. Lowy, Pathogenesis of methicillin-resistant *Staphylococcus aureus* infection. Clin. Infect. Dis . 46 (2008) S350-S359.

[18] L.A. Cohn, J.R. Middleton, A veterinary perspective on methicillin-resistant staphylococci. J Vet Emerg Crit Care. 20 (2010) 31-45.

[19] J.S. Kim, W. Song, H.S. Kim, et al., Association between the methicillin resistance of clinical isolates of *Staphylococcus aureus,* their staphylococcal cassette chromosome *mec* (SCC*mec*) subtype classification, and their toxin gene profiles. Diagn Microbiol Infect Dis. 56 (2006) 289-295.

[20] T. Ferry, D. Thomas, A.L. Genestier, et al., Comparative prevalence of superantigen genes in *Staphylococcus aureus* isolates causing sepsis with and without septic shock. Clin. Infect. Dis. 41 (2005) 771-777.

[21] B.J. Berube, J. Bubeck Wardenburg, *Staphylococcus aureus* alpha-toxin: nearly a century of intrigue. Toxins. 5 (2013) 1140-166.

[22] D.P. Kateete, S. Namazzi, M. Okee, et al., High prevalence of methicillin resistant *Staphylococcus aureus* in the surgical units of Mulago hospital in Kampala, Uganda. BMC Res Notes. 4 (2011) 326.

[23] S.K. Shukla, M.E. Karow, J.M. Brady, et al., Virulence genes and genotypic associations in nasal carriage, community-associated methicillin-susceptible and methicillin-resistant USA400 *Staphylococcus aureus* isolates. J. Clin. Microbiol. 48 (2010) 3582-3592.

[24] N. Rusenova, W. Gebreyes, M. Koleva, et al., Comparison of three methods for routine detection of *Staphylococcus aureus* isolated from bovine mastitis, Kafkas Univ Vet Fak Derg. 19 (2013) 709-712.

[25] H. Rohde, E.C. Burandt, N. Siemssen, et al., Polysaccharide intercellular adhesin or protein factors in biofilm accumulation of *Staphylococcus epidermidis* and *Staphylococcus aureus* isolated from prosthetic hip and knee joint infections. Biomaterials. 28 (2007) 1711-1720.

[26] A. Tristan, L. Ying, M. Bes, et al., Use of multiplex PCR to identify *Staphylococcus aureus* adhesins involved in human hematogenous infections. J. Clin. Microbiol. 41 (2003) 4465-4467.

[27] A. Ghasemian, S. Najar Peerayeh, B. Bakhshi, et al., The Microbial Surface Components Recognizing Adhesive Matrix Molecules (MSCRAMMs) Genes among Clinical Isolates of *Staphylococcus aureus* from Hospitalized Children. Iran J Pathol. 10 (2015) 258-264.

[28] T. Zmantar, K. Chaieb, H. Makni, et al., Detection by PCR of adhesins genes and slime production in clinical Staphylococcus aureus. J. Basic. Microbiol. 48 (2008) 308-314.

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

epidemic cluster in isolates from orthopedic implant infections. Biomaterials. 30 (2009) 6621-6628.

[30] S. Nitzsche, C. Zweifel, R. Stephan, Phenotypic and genotypic traits of *Staphylococcus aureus* strains isolated from pig carcasses. Vet. Microbiol. 120 (2007) 292-299.

[31] E. Vautor, G. Abadie, A. Pont, et al., Evaluation of the presence of the bap gene in *Staphylococcus aureus* isolates recovered from human and animals species. Vet. Microbiol. 127 (2008) 407-411.

[32] B. Khoramian, F. Jabalameli, A. Niasari-Naslaji, et al., Comparison of virulence factors and biofilm formation among *Staphylococcus aureus* strains isolated from human and bovine infections. Microb Pathog. 88 (2015) 73-77.

[33] G. Paniagua-Contreras, E. Monroy-Perez, R. Gutierrez-Lucas, et al., Genotypic characterization of methicillin-resistant *Staphylococcus aureus* strains isolated from the anterior nares and catheter of ambulatory hemodialysis patients in Mexico. Folia Microbiol. 59 (2014) 295-302.

[34] M. Otto. MRSA virulence and spread. Cell Microbiol. 14 (2012) 1513–1521.

Table1. PCR  primers  and  annealing temperature for toxin genes

| Gene | Primer Sequence (5' to 3') | Product Size, bp | Annealing temperature (°C) | Reference |
|------|---------------------------|------------------|----------------------------|-----------|
| *sea* | F- GGT TAT CAA TGT GCG GGT GG<br>R- CGG CAC TTT TTT CTC TTC GG | 102 | 57 | |
| *seb* | F- GTA TGG TGG TGT AAC TGA GC<br>R-CCA AAT AGT GAC GAG TTA GG | 164 | 57 | |
| *sec* | F- AGA TGA AGT AGT TGA TGT GTA TGG<br>R- CAC ACT TTT AGA ATC AAC CG | 451 | 57 | |
| *eta* | F-GCA GGT GTT GAT TTA GCA TT<br>R-AGA TGT CCC TAT TTT TGC TG | 93 | 53 | |
| *tst* | F-ACC CCT GTT CCC TTA TCA TC<br>R-TTT TCA GTA TTT GTA ACG CC | 326 | 53 | 5 |
| *hla* | F:  CTG ATT ACT ATC CAA GAA ATT CGA TTG<br>R:  CTT TCC AGC CTA CTT TTT TAT CAG T | 209 | 54 | |
| *hlb* | F:GTG CAC TTA CTG ACA ATA GTG C<br>R:GTT GAT GAG TAG CTA CCT TCA GT | 309 | 54 | |
| *pvl* | F:ATCATTAGGTAAAATGTCTGGACATGATCCA<br>R:GCATCAASTGTATTGGATAGCAAAAGC | 432 | 55 | |

13

**Table2**. primers and annealing temperature for MSCRAMMs and biofilm genes

| Gene | Primer Sequence (5' to 3') | Product Size, bp | Annealing temperature (°C) | Reference |
|------|----------------------------|------------------|----------------------------|-----------|
| *cna* | F:GTCAAGCAGTTATTAACACCAGAC<br>R:AATCAGTAATTGCACTTTGTCCACTG | 423 | 55 | |
| *eno* | F:ACGTGCAGCAGCTGACT<br>R: CAACAGCATCTTCAGTACCTTC | 302 | 55 | |
| *fib* | F: CTACAACTACAATTGCGTCAACAG<br>R: GCTCTTGTAAGACCATTTTCTTCAC | 404 | 55 | |
| *fnbA* | F:GTGAAGTTTTAGAAGGTGGAAAGATTAG<br>R:GCTCTTGTAAGACCATTTTTCTTCAC | 643 | 55 | 10 |
| *fnbB* | F:GTAACAGCTAATGGTCGAATTGATACT<br>R: CAAGTTCGATAGGAGTACTATGTTC | 524 | 55 | |
| *ebpS* | F: CATCCAGAACCAATCGAAGAC<br>R: AGTTACATCATCATGTTTATCTTTTG | 185 | 55 | |
| *icaA* | F: TGG CTG TAT TAA GCG AAG TC<br>R: CCT CTG TCT GGG CTT GAC C | 669 | 55 | |
| *icaD* | F: ATGGTCAAGCCCAGACAGAG<br>R: AGTATTTTCAATGTTTAAAGCAA | 198 | 55 | |

14

**Table3**. Distribution of toxin genes  among MRSA isolates with SCC*mec* type IIIA

| Toxin Genes | N(%) of isolates |
|---|---|
| *sea* | 7 (12.9) |
| *seb* | 1 (1.8) |
| *Sea+hla* | 20(37) |
| *Sea+hlb* | 1(1.8) |
| *hla+hlb* | 1(1.8) |
| *Sea+hla+hlb* | 7(12.9) |

15

**Table4**. Distribution of adhesion and biofilm genes among MRSA isolates with SCC*mec* type IIIA

| Adhesion genes | N(%) of isolates |
| --- | --- |
| *cna* | 1(1.8) |
| *fib* | 1(1.8) |
| *eno* | 2(3.7) |
| *icaA* | 1(1.8) |
| *fib+eno* | 5(9.2) |
| *eno+icaA* | 2(3.7) |
| *fib+icaA* | 1(1.8) |
| *eno+icaD* | 1(1.8) |
| *cna+fib+eno* | 2(3.7) |
| *fib+ eno+icaA* | 4(7.4) |
| *fib+eno+icaA+icaD* | 11(20.3) |
| *cna+ fib+eno+icaA* | 1(1.8) |
| *cna+ fib+eno+fnbA+ icaA* | 1(1.8) |
| *cna+ fib+eno+fnbB+ icaA* | 1(1.8) |
| *cna+ fib+eno+fnbB+ icaA+icaD* | 2(3.7) |
| *cna+ fib+eno+fnbA+ icaA+icaD* | 1(1.8) |

16

- A total of 128 *S. aureus* isolates from burn wound infection were analyzed.

- 54 isolates were identified as MRSA with SCC*mec* 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.