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Original Article

SCC*mec* Typing and the Association of *pvl*, *ACME*, *sea* and *seb* Genes in *Staphylococcus aureus* Isolates From Burn Wound Infections

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Abstract

Background: *Staphylococcus aureus* is a very important hospital and community-acquired pathogen that is prevalent in burn wounds, so finding the sources of contamination and infection with it are important for its control. The aim of this study was to do SCC*mec* typing and determine the prevalence of *pvl, ACME*, *sea* and *seb* genes among *S. aureus* isolates from burn patients.

Methods: Fifty *S. aureus* isolates were sampled from burn patients and were identified. These isolates were typed based on SCC*mec* typing and detection of *mec*A and *pvl* genes using multiplex polymerase chain reaction (PCR) method and also were screened for the presence of *ACME*, *sea* and *seb* genes by PCR.

Results: Three isolates were identified as methicillin-resistant strains. The *sea* gene was identified in 22% of isolates while *pvl*, *ACME* and *seb* genes were not found in the isolates**.** Multiplex PCR SCC*mec* typing of 3 methicillin resistant *S. aureus* (MRSA) isolates showed that 2 isolates belong to SCC*mec* type II and 1 isolate to type I.

Conclusions: The results suggest that methicillin resistant *S. aureus* isolates are prevalent in burn patients and *mec*A gene is involved in resistance to methicillin. These strains belonged to SCC*mec* type II and I which can affect horizontal gene transfer among *S. aureus* isolates and hence distribution of virulence genes. Therefore, it is necessary to frequently monitor *S. aureus* isolates through typing and screening virulence genes to control this pathogen.

Keyword: *Staphylococcus aureus*, *SCCmec* typing, *pvl*, *ACME,* Enterotoxins, *sea*, *seb*

Background

Staphylococcus aureus is a very important hospital and community-acquired pathogen that causes extended-spectrum infections. The infectious potential is related to various bacterial surface cell components and extracellular secreting proteins (1). Various factors such as toxins, invasion and antibiotic resistance are involved in pathogenicity of this bacterium. Resistant strains against a wide variety of antimicrobial agents are emerging frequently due to expression of new resistance mechanisms by this pathogen (2,3). Methicillin resistant *S. aureus* (MRSA) strains are the main resistant strains of this pathogen around the world and vancomycin is the only choice to fight them. But unfortunately, vancomycin resistant strains are also growing among hospital and/or community-acquired infections (4). The *mec*A gene on the staphylococcal cassette chromosome *mec* (SCCmec) element is responsible for methicillin resistance (5). SCC*mec* genomic island contains *mec* gene complex and *ccr* (cassette chromosome recombinase) gene complex**.** Eleven major types (I– XI) are known for SCC*mec* elements some of which are divided into subtypes. In cases of hospital-acquired MRSA (HA-MRSA), more frequently detected SCC*mec* types have been I, II, and III, while in community-acquired MRSA (CA-MRSA), the types IV and V have been mostly involved (5). Detection of the SCC*mec* type of an isolate is necessary for control of hospital infections and prevention of the transmission of infections (6).

The arginine catabolic mobile element (*ACME*) inhibits polymorphonuclear cell production and plays an important role in bacterial growth and survival, colonization in human skin and extensive dissemination. In MRSA strains, *ACME* is always integrated with SCC*mec* elements. SCC*mec* recombinase is likely to mediate its integration and excision (4). Physical relationship between *SCCmec* and *ACME* genes suggest that the pathogenicity and antibiotic resistance of this pathogen are related to each other (7).

Panton-Valentine leukocidin (PVL), a pore-forming cytotoxin that damages membranes of host defense cells, is encoded by 2 adjacent open reading frames (LukS-PV, LukF-PV) (3,8). Presence of *pvl* is considered as a genetic marker for MRSA strains, but fortunately PVL is only produced by less than 5% of HA-MRSA and CA-MRSA (3).

Staphylococcus aureus produces a group of 21 staphylococcal enterotoxins (SEs) that are characterized by high thermostability and resistance to most proteolytic enzymes and also various environmental conditions (6). The genes *sea* and *see* are carried by a temperate bacteriophages and *seb* and *sec* genes are located on chromosomes. SEA is one of the most important causes of gastroenteritis. In staphylococcal scalded-skin syndrome, the ETA and ETB enterotoxins are involved jointly or separately. Therefore, screening of *S. aureus* isolates for SEs is necessary in order to gain knowledge about their prevalence and enterotoxigenicity potential (1,6).

Burn patients are immunocompromised and hence exhibit more susceptibility to infections especially *S. aureus* infection (9). Therefore, due to the prevalence of infections caused by *S. aureus* strains in burn patients, it is important to find the source of contamination with it to control the infection.

Objectives

The aim of the study was to do SCC*mec* typing and detect *pvl, ACME*, *sea* and *seb* genes in *S. aureus* isolates from patients admitted to a burn hospital in Ahvaz.

Materials and Methods

Bacterial Strains

Fifty *S. aureus* isolates previously collected from burn patients in Taleghani hospital, Ahvaz, southwest of Iran were included in this study. The methicillin resistance of isolates was investigated by Mueller- Hinton agar screening test according to the CLSI (10).

DNA Extraction

Pure colonies suspension was boiled (15 min, 100°C), centrifuged (1 minute, 5000 rpm) and 500 µL of cold ethanol was added to 200 µL of cultured supernatant. This mixture was kept at -20°C for 1 hour and then centrifuged (10 minutes, 13000 rpm). The precipitate was air-dried at 37°C and dissolved in 50 µL sterile deionized water (11).

Polymerase Chain Reaction Assays

Multiplex polymerase chain reaction (PCR) assay was used for detection of *mec*A (310bp) and *pvl* (433bp) genes using primers listed in Table 1. PCR was carried out using 2 µL of DNA, forward and reverse primers (10 ρM) for *mec*A and *pvl* primers, 12.5 µL of 2X Amplicon Master Mix and miliQ water up to 25 µL. Sterile water and DNA extracted from a MRSA strain were used as negative and positive controls, respectively. The amplification was performed at denaturation (94°C, 10 minutes), 10 cycles each consisting of denaturation (94°C, 45 seconds), annealing (55°C, 45 seconds), and extension (72°C, 75 seconds) followed 25 cycles with 50°C annealing temperature and a final extension step (72°C, 10 minutes) (12).

Screening of *ACME* (1941bp), *sea* (102 bp) and *seb* (164 bp) genes was performed by PCR assay separately. Each reaction contained 2 µL of template DNA, 0.4 ρM of each forward and reverse primers, and 12.5 µL of 2X Amplicon Master Mix and miliQ water up to 25 µL. Controls were also regarded. A PCR protocol was conducted as previously described with 55°C annealing temperature. All experiments were done in duplicate to confirm their reproducibility (9).

A multiplex PCR assay was also used for typing the SCC*mec* types I to V (Tables 2 and 3) as cycling program previously described for multiplex PCR (13).

Results

In this study, 50 *S. aureus* isolates were studied all of which were resistant to methicillin in antibiotic susceptibility test and were regarded as MRSA.

The amplification of SE *sea* was successful in 11 of 50

Table 1. The Primer Sequence and Amplicon Size of the Understudy Genes

Gene	Primer	Oligonucleotide Sequence (5′→3′)	Amplicon Size (bp)	Reference
mecA	*mec*A1-F *mec*A2-R	GTAGAAATGACTGAACGTCCGATAA CCAATTCCACATTGTTTCGGTCTAA	310	(11)
sea	GSEAR-1 GSEAR-2	GGTTATCAATGTGCGGGTGG CGGCACTTTTTTCTCTTCGG	102	(9)
seb	GSEBR-1 GSEBR-2	GTATGGTGGTGTAACTGAGC CCAAATAGTGACGAGTTAGG	164	(9)
pvl	Luk-PV-1F Luk-PV-2R	ATCATTAGGTAAAATGTCTGGACATGATCCA GCATCAAGTGTATTGGATAGCAAAAGC	433	(11)
ACME	AIPS-27 AIPS-28	CTAACACTGAACCCCAATG GAGCCAGAAGTACGCGAG	1941	(7)

Table 2. Amplicon Size of SCC*mec* Types

SCCmec types	Oligonucleotide sequence (5' → 3')	Amplicon Size (bp)	CAMRSA/HA-MRSA
Type I-F Type I-R	GCTTTAAAGAGTGTCGTTACAGG GTTCTCTCATAGTATGACGTCC	613	HA-MRSA
Type II-F Type II-R	CGTTGAAGATGATGAAGCG CGAAATCAATGGTTAATGGACC	398	HA-MRSA
Type III-F Type III-R	CCATATTGTGTACGATGCG CCTTAGTTGTCGTAACAGATCG	280	HA-MRSA
Type IVa-F Type IVa-R	GCCTTATTCGAAGAAACCG CTACTCTTCTGAAAAGCGTCG	776	CA-MRSA
Type IVb-F Type IVb-R	TCTGGAATTACTTCAGCTGC AAACAATATTGCTCTCCCTC	493	CA-MRSA
Type IVc-F Type IVc-R	ACAATATTTGTATTATCGGAGAGC TTGGTATGAGGTATTGCTGG	200	CA-MRSA
Type IVd-F5 Type IVd-R6	CTCAAAATACGGACCCCAATACA TGCTCCAGTAATTGCTAAAG	881	CA-MRSA
Type V-F Type V-R	GAACATTGTTACTTAAATGAGCG TGAAAGTTGTACCCTTGACACC	325	CA-MRSA

Table 3. Reagents used for Multiplex PCR of SCC*mec* Types

Material	Stock Concentration	Final	Vol.
Master mix	2X	1X	12.5 µL
Taq polymerase	5 u/µL	1.5 u	0.3 µL
$MgCl_2$	50 mM	1.5 mM	0.75 µL
Primers II, III, V I, IVa, IVb I, IVd, IVc	10 pmol/µL	0.4 pmol/µL	6 µL each primer 1 µL
DNA	-	-	5 µL
DEPC water	-	-	0.45 µL
Final volume	-	-	25 µL

In our study, we used multiplex PCR method for detection of *mec*A and *pvl* genes in50 *S. aureus* isolates. The *mec*A gene was detected in 6% of the *S. aureus* isolates but *pvl* gene was not found in any isolates. Holmes *et al.*

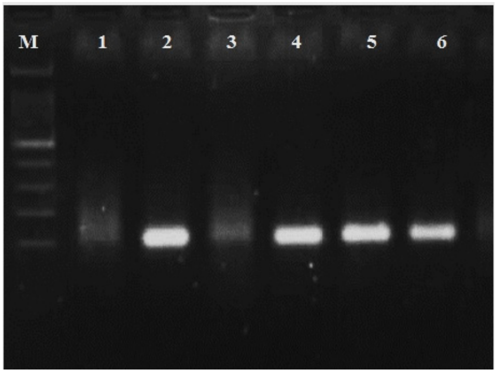

Figure 1. Electrophoresis of PCR Products of Sea (102 bp) Amplification on 1.5% Agarose Gel.
M: 100 bp molecular weight ladder; lane1: negative control; lanes 2: positive control, lane 3: negative sea in clinical isolates, Lane 4, 5 and 6: positive sea in clinical isolates.

isolates (22%) (Figure 1). Furthermore, PCR amplification in 3 samples (6%) was positive for *mec*A gene (Figure 2). All of the *mec*A positive strains were also positive for *sea* gene. Regarding *pvl, ACME* and *seb* genes, none of the tested samples were positive for these genes.

In SCC*mec* typing, the amplification revealed 2 main SCC*mec* types including SCC*mec* types I and II, yielding only one band in the multiplex PCR. Two of them were SCC*mec* types II (398 bp) and one of them was SCC*mec* types I (613 bp) (Figure 3).

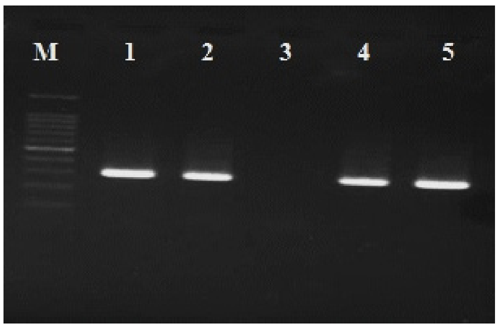

Figure 2. Electrophoresis of PCR Products of *mec*A (310 bp) Amplification on 1.5% Agarose Gel.
M: 100bp molecular weight marker, lane 1: positive control for mecA, lane 2, 4 and 5: positive mecA in clinical isolates; lane 3: negative control (distilled water).

Discussion

Staphylococcus aureus is one of the most important agent of hospital-acquired infections that can cause even life-threatening infections (6). It is therefore necessary to frequently monitor its prevalence and virulent and resistance markers so as to find the source of infection with it and plan for control programs to limit its spread. Conventional methods such as bacterial culture and biochemical testes cannot detect accurately the prevalence of resistant strains especially MRSA while molecular methods can be used to rapidly and reliably achieve this purpose.

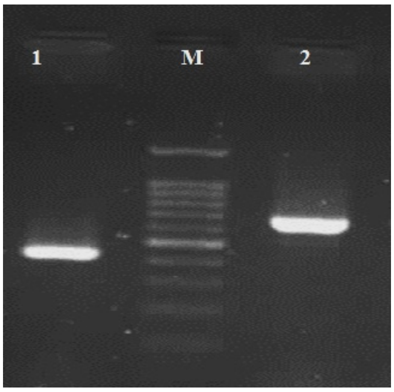

Figure 3. Electrophoresis of PCR products from Amplification of SCC*mec* Types.
Lane 1: SCC*mec* type II (398 bp); M: 100 bp molecular weight marker; lane 2: SCC*mec* type I (613 bp)

(2005) reported prevalence of *pvl* to be 1.6% (8 of 515) (2). In the study of Okon et al, the prevalence of *mec*A gene among 96 MRSA isolates was reported to be 12.5%, but none of positive *mec*A isolates was positive for *pvl* gene (14).

Khosravi et al detected the presence of *mec*A and *pvl* genes in, respectively, 87% and 7% of *S. aureus* strains isolated from burn hospital in Ahvaz (15).

The results of Kim et al showed that from 100 *S. aureus* isolates, only 3 isolates carried the *mec*A gene and 1 carried the *pvl* gene (16).

The prevalence of *mec*A gene among *S. aureus* isolates in this study was lower than those observed in other studies. This could be explained by the low number of *S. aureus* isolates or the different origin of isolates in other studies in Iran. Furthermore, it may be due to that other mobile genetic elements, such as plasmids, transposons and phages, contain resistance determinants, and therefore their elimination from bacterial cell would result in the absence of *mecA* gene and consequently no association with *pvl* gene (17). In our research, *pvl* gene was not found in any isolates, which is in agreement with the results of other studies.

Multiplex PCR assay for SCC*mec* typing of 3 MRSA isolates showed that 2 isolates belonged to SCC*mec* type II and 1 isolate to type I. SCC*mec* types I, II, and III are dominant among HA-MRSA strains and are multidrug resistant, but SCC*mec* types IV, V and VI have been associated more frequently with CA-MRSA strains and have been frequently reported to be susceptible to most antibiotics except beta-lactam antibiotics (5).

In the study of Boye et al, 98% of isolates were typed by the multiplex PCR assay. SCC*mec* type IV was the most common type (84%), followed by type V (6%), type I (4%) and type II (3%). SCC*mec* type III was found only in three isolates (18).

The results of Zeinali et al on 58 MRSA strains revealed that SCC*mec* type II was the most common type, followed by type IVb, type IVd, type I and type V (6).

In the study of Budimiri et al, out of 77 MRSA isolates, type I was the most frequently detected, followed by type II and type III (19). Namvar et al studied 40 isolates of *S. aureus* collected from burn patients in Tehran, Iran. Based on the multiplex PCR assay, five different SCC*mec* types (type III: 47.5%; type IV: 25%; type V: 10%; type II: 10%; and type I: 7.5%) were detected (20).

In the study done by Kim et al, 100 *S. aureus* isolates were studied, 3 MRSA samples were found as *mec*A positive and 3 different SCC*mec* types were detected as type I, IV, V (16).

ACME is a large genetic region that is observed in MRSA isolates especially MRSA USA300 clone (21). In our study this gene was not detected in all tested samples. Shore et al reported the prevalence of *ACME* gene in 238 *S. aureus* isolates as 9.7% (22). All of the isolates in the study of Marquez et al were negative for this gene (23).

Detecting *se* genes by molecular techniques could help understanding the virulence mechanisms and pathogenicity potential of *S. aureus*. In our study, 22% of isolates were positive for *sea* and none of the isolates were positive for *seb* gene.

In the study performed by Nashev et al, 23% of isolates were positive for *sea* gene (24). Saadati et al reported the prevalence of the *sea* gene as being 5% (1). These results are in agreement with our study.

The prevalence of *seb* gene in the studies of Ferry et al and Lovseth et al in Brazil was reported to be 86 % and 14.3% , respectively (25,26).

Rezaei et al studied 200 *S. aureus* isolates, 60 (30%) of which carried *sea* (4). In 2 other similar studies, the prevalence of *sea* gene was reported as being 74% and 46.9%, respectively (27). These results are in agreement with our results. However, differences in the results of various studies can be related to the source of sampling, geographical origin, sensitivity of identification methods and the quantity of samples that can affect the prevalence (28). Moreover, the incidence rate of SEA is higher than those of the other SEs, indicating the greater importance of this type of SE than others.

Conclusions

Taken together, detecting the *sea* genes by molecular techniques could help understanding the virulence factors of prevalent *S. aureus* isolates. The data presented in this study represent the information about the prevalence of methicillin resistant and enterotoxigenic *S. aureus* isolates from patients in Ahvaz (southwest of Iran) and highlight an urgent need for epidemiological studies to monitor the distribution of these virulent factors among clinical isolates of *S. aureus*.

Ethical Approval

None to be declared.

Conflict of Interest Disclosures
The authors declare that they have no conflict of interests.

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