SCCmec 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 SCCmec 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 SCCmec typing and detection of mecA 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 pvl gene was identified in 22% of isolates while pvl, ACME and seb genes were not found in the isolates. Multiplex PCR SCCmec typing of 3 methicillin resistant S. aureus (MRSA) isolates showed that 2 isolates belong to SCCmec type II and 1 isolate to type I.

Conclusions: The results suggest that methicillin resistant S. aureus isolates are prevalent in burn patients and mecA gene is involved in resistance to methicillin. These strains belonged to SCCmec 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 mecA gene on the staphylococcal cassette chromosome mec (SCCmec) element is responsible for methicillin resistance (5). SCCmec genomic island contains mec gene complex and ccr (cassette chromosome recombinase) gene complex. Eleven major types (I–XI) are known for SCCmec elements some of which are divided into subtypes. In cases of hospital-acquired MRSA (HA-MRSA), more frequently detected SCCmec 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 SCCmec 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 SCCmec elements. SCCmec 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 SCCmec 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 mecA (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 pM) for mecA 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 pM 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 SCCmec 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

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer</th>
<th>Oligonucleotide Sequence (5’→3’)</th>
<th>Amplicon Size (bp)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>mecA</td>
<td>mecA1-F</td>
<td>GTAGAAATGACTGAACGTCCGATAACCAATTCCACATTGTTTCGGTCTAA</td>
<td>310</td>
<td>(11)</td>
</tr>
<tr>
<td></td>
<td>mecA2-R</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sea</td>
<td>GSEAR-1</td>
<td>GGTATATCAATGTGCGGCTGGCGCCACCTTTTCTCTC</td>
<td>102</td>
<td>(9)</td>
</tr>
<tr>
<td></td>
<td>GSEAR-2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>seb</td>
<td>GSEBR-1</td>
<td>GTATGCGTGTGTAACGTGACCCAAATGTGAG</td>
<td>164</td>
<td>(9)</td>
</tr>
<tr>
<td></td>
<td>GSEBR-2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pvl</td>
<td>Luk-PV-1F</td>
<td>ATCATGGTAAATGTGCGGACATGCACGCAT</td>
<td>433</td>
<td>(11)</td>
</tr>
<tr>
<td></td>
<td>Luk-PV-2R</td>
<td>GCAATGTCGATGCGAGAGAAG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACME</td>
<td>AIPS-27</td>
<td>CTAACACTGAACCCCAATG GAGCCGAAAGTACCGG</td>
<td>1941</td>
<td>(7)</td>
</tr>
</tbody>
</table>
SCCmec Typing and the Association of pvl, ACME, sea and seb Genes

In our study, we used multiplex PCR method for detection of mecA and pvl genes in 50 S. aureus isolates. The mecA gene was detected in 6% of the S. aureus isolates but pvl gene was not found in any isolates. Holmes et al.

Table 2. Amplicon Size of SCCmec Types

<table>
<thead>
<tr>
<th>SCCmec types</th>
<th>Oligonucleotide sequence (5’ → 3’)</th>
<th>Amplicon Size (bp)</th>
<th>CAMRSA/HA-MRSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type I-F</td>
<td>GCTTTAAAGTTCGTACAGG</td>
<td>613</td>
<td>HA-MRSA</td>
</tr>
<tr>
<td>Type I-R</td>
<td>GTTCCTTCTACGTAGAGG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type II-F</td>
<td>CGTGAAGATAGAGAAGG</td>
<td>398</td>
<td>HA-MRSA</td>
</tr>
<tr>
<td>Type II-R</td>
<td>CGAAAGCTAAGTGAAGATGA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type III-F</td>
<td>CATATTGATGCATGACG</td>
<td>280</td>
<td>HA-MRSA</td>
</tr>
<tr>
<td>Type III-R</td>
<td>CTCTACGTAGAAGAGACG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type IVa-F</td>
<td>GCTTTATCGAAGAAAAAGGG</td>
<td>776</td>
<td>CA-MRSA</td>
</tr>
<tr>
<td>Type IVa-R</td>
<td>CTATCTCTTCTGAAAAAGCCG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type IVb-F</td>
<td>TCTGGAATCTTCTAGCTGC</td>
<td>493</td>
<td>CA-MRSA</td>
</tr>
<tr>
<td>Type IVb-R</td>
<td>AAAACATAGGCTGCTCCCTC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type IVc-F</td>
<td>ACAATTTGATATACGAGAAGGC</td>
<td>200</td>
<td>CA-MRSA</td>
</tr>
<tr>
<td>Type IVc-R</td>
<td>TTGGTATAGTTAGTTGCTAAG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type IVd-F5</td>
<td>CTCAAAAATCCGACCCTAATACAAAA</td>
<td>881</td>
<td>CA-MRSA</td>
</tr>
<tr>
<td>Type IVd-R6</td>
<td>TGCTCCAGTAATGCTAAG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type V-F</td>
<td>GAAATTAGTTACATGGATGACG</td>
<td>325</td>
<td>CA-MRSA</td>
</tr>
<tr>
<td>Type V-R</td>
<td>TGAAGTTGATACCTTGGACCC</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3. Reagents used for Multiplex PCR of SCCmec Types

<table>
<thead>
<tr>
<th>Material</th>
<th>Stock Concentration</th>
<th>Final Vol.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Master mix</td>
<td>2X</td>
<td>1X</td>
</tr>
<tr>
<td>Taq polymerase</td>
<td>5 u/μL</td>
<td>1.5 u</td>
</tr>
<tr>
<td>MgCl₂</td>
<td>50 mM</td>
<td>1.5 mM</td>
</tr>
<tr>
<td>Primers II, III, V</td>
<td>10 pmol/μL</td>
<td>0.4 pmol/μL</td>
</tr>
<tr>
<td>DNA</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>DEPC water</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Final volume</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

isolates (22%) (Figure 1). Furthermore, PCR amplification in 3 samples (6%) was positive for mecA gene (Figure 2). All of the mecA 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 SCCmec typing, the amplification revealed 2 main SCCmec types including SCCmec types I and II, yielding only one band in the multiplex PCR. Two of them were SCCmec types II (398 bp) and one of them was SCCmec types I (613 bp) (Figure 3).

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 tests cannot detect accurately the prevalence of resistant strains especially MRSA while molecular methods can be used to rapidly and reliably achieve this purpose.
Electrophoresis of PCR products from Amplification of mecA isolates, 60 (30%) mecA positive and 3 different SCCmec 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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References
25. Ferry T, Thomas D, Genestier AL, Bes M, Lina G, Vandenesch

