Diversity of the SCCmec types among *Staphylococcus epidermidis* clinical isolates from intensive care unit patients

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**ABSTRACT**

**Objective:** To determine different types of staphylococcal cassette chromosome mec (SCCmec) in *Staphylococcus epidermidis* (S. epidermidis) clinical isolates from intensive care unit patients, Tehran, Iran.

**Methods:** A total of 59 *S. epidermidis* strains were isolated from intensive care unit patients in Tehran, Iran. Isolates of *S. epidermidis* were identified by conventional biochemical diagnostic tests. The PCR was used to detect the mecA-positive isolates and type the ccr and mec complexes.

**Results:** The mecA gene was amplified in 91.5% of isolates. Moreover, the predominant SCCmec type was the SCCmec type III which was detected in 53.7% of isolates. The frequencies of other SCCmec types were as following: 5.6% in SCCmec type I, 3.7% in SCCmec type II, 5.6% in SCCmec type IV, 7.4% in SCCmec type V and 3.7% in SCCmec type VII. Furthermore, 20.3% of tested isolates were not typeable using the specific primers.

**Conclusions:** SCCmec type III was the most frequent SCCmec type in this study. Eleven of 54 SCCmec types were not classified, among which one isolate possessed mec type A but the ccr type cannot be distinguished with using primers. The frequency of SCCmec type VII was 3.7%, which was reported in *S. epidermidis* for the first time.

**1. Introduction**

The cause of methicillin resistance in staphylococci is the mecA gene, carried by several mobile genetic elements called staphylococcal cassette chromosome mec (SCCmec) elements. The origin of SCCmec remains unknown, but it seems that it has the ability to transmit horizontally among *Staphylococci* strains. When this chromosomal cassette enters the genome of a staphylococcal strain, the methicillin resistance occurs due to the expression of penicillin binding protein 2a. This cassette chromosome is divided into several types and subtypes based on the differences in the structure and genetic content [1,2]. Different types of SCCmec are classified based on the combination of mec and ccr gene complexes. The ccr genes encode recombinase enzymes which are required for the entry and integration of the cassette to the bacterial chromosome and thus they can lead to the mobility of chromosomal cassette. So far, three ccr genes have been identified in *Staphylococcus aureus* (S. aureus); ccrA, ccrB and ccrC. Two distinct groups of ccr genes have been reported: a group that carries two ccr genes (ccrA, ccrB) and a group that carries one ccr gene (mec complex). The ccrC is composed of mecA and its regulatory genes, including mecI and mecR1, the additional sequences (IS 1272, IS 431) and the variable regions (hypervariable region). On the other hand, five types of mec complexes have been identified in *S. aureus* including A, B, C1, C2, and E. The interstitial regions around the ccr and mec gene complexes are called J regions that may carry other antibiotic resistance genes. So far, 11 SCCmec types have been identified in *S. aureus*, and coded as 1-XI. Another denomination is also used by International Working Group on the Classification of Staphylococcal Cassette Chromosome Elements and is indicated in the form of 1B, 2A, 2B, 3A, 4B, 5C2, 5C1, etc., which is a combination of the number of ccr complex and the mec complex types (2,3). Several studies have suggested that the structural heterogeneity of this chromosomal cassette in clinical isolates of coagulase-negative methicillin-resistant *Staphylococci* is higher than that of methicillin-resistant *S. aureus*. Several clinical isolates of methicillin-resistant coagulase-negative *Staphylococcal* cannot be classified in the known categories because they have a new combination of chromosomal cassette that has not been reported so far and the PCR has not been performed for ccr gene, or there are more than one ccr types. Previous studies have clarified that the diversity of the ccr gene complex is more than mec complex. A variety of unclassified types and the types with more than one ccr gene are more observed in coagulase-negative *Staphylococci*. The coagulase-negative *Staphylococci* SCCmec types exhibit resistance
2. Materials and methods

2.1. Bacterial isolates

This was a cross-sectional study. The total of 59 *S. epidermidis* strains were isolated from blood (n = 50), urine (n = 4), trachea (n = 4) and wound (n = 1) samples from ICU patients in Tehran, Iran. The isolates were identified by conventional bacteriological tests such as enrichment in tryptic soy broth, colony morphology and growth on manitol salt agar without manitol and growth on sheep blood agar, tube coagulase, catalase, carbohydrate tests such as enrichment in tryptic soy broth, colony morphology and growth on manitol salt agar without manitol fermentation[4,5].

2.2. DNA extraction

DNA of each *S. epidermidis* isolate was extracted from 1 mL of overnight bacterial culture. Extraction was performed by adding 10 mmol/L tris and 1 mmol/L ethylene diamine tetraacetic acid (pH = 8) buffer and lysostaphin, 95 °C for 5 min, then 0 °C for 5 min, and centrifuged in 10,000 r/min for 5 min. The supernatant was used as the template DNA in PCR reactions. DNA was measured using a BioPhotometer (Eppendorf, Germany) to determine the concentration and purity.

2.3. Different types of SCCmec identification

In order to identify different types of SCCmec, the presence of *mecA* was investigated in all isolates, then SCCmec type I-V, *ccr* type and mec complex class were investigated in *mecA*-positive strains. Primers and PCR condition were shown in Table 1.

2.4. Statistical analysis

Confidence interval (CI) test was used to assess the statistical significance with confidence level of 95% (α = 0.05).

3. Results

In this study, frequency of *mecA* was 91.5% (95% CI: 84.8–98.2). Of the 54 *mecA*-positive isolates, the most frequent SCCmec type was SCCmec type III which was observed in 53.7% (95% CI: 40.4–67.0) of isolates. Frequencies of other SCCmec types were as following: 5.6% (95% CI: 0.0–11.7) in SCCmec type I, 3.7% (95% CI: 0.0–8.7) in type II, 5.6% (95% CI: 0.0–11.7) in type IV, 7.4% (95% CI: 0.4–14.4) in type V and 3.7% (95% CI: 0.0–8.7) in type VII. Of the 54 *mecA*-positive isolates, 20.3% were non-classified with primers used in this study. One isolate harbored mec type A and carried undefined *ccr* type and likewise one isolate harbored mec type B with undefined *ccr* type, also one isolate harbored...

Table 1

| Target       | Forward 5 → 3/ | Reverse 5 → 3  | PCR condition Expected size (bp) | Reference |
|--------------|----------------|----------------|----------------------------------|-----------|
| *mecA*       | GTGAAAGATATACCAAGTGAAT/ | ATGCCTATAGATTAGGAGAT | 4 min, 94 °C; 35 cycles of 1 min, 94 °C; 1 min 57 °C; 2 min 30 s, 94 °C; final extension, 2 min, 72 °C | 147 [6]    |
| SCCmec type I | GTTCTTATAGATCTGACGGG/ | GTTCTCATAGATGACGGTGCC | 5 min, 94 °C; 30 cycles of 1 min, 94 °C; 1 min 51 °C; 2 min, 72 °C; final extension, 2 min, 72 °C | 398 [6]    |
| SCCmec type II | CGTTGAAAGATGATGAGCG/ | CGAAATCAGGGTTAATGACC | 5 min, 94 °C; 30 cycles of 1 min, 94 °C; 1 min 51 °C; 2 min, 72 °C; final extension, 2 min, 72 °C | 613 [6]    |
| SCCmec type III | CCTATTTGGTACGATGGC/ | CCTATTTGGTACGATGGC | 5 min, 94 °C; 30 cycles of 1 min, 94 °C; 1 min 51 °C; 2 min, 72 °C; final extension, 2 min, 72 °C | 280 [6]    |
| SCCmec type IVa | GTCTCTTGTCAAGAAGGTTG | GTCTCTTGTCAAGAAGGTTG | 5 min, 94 °C; 30 cycles of 1 min, 94 °C; 1 min 51 °C; 2 min, 72 °C; final extension, 2 min, 72 °C | 776 [6]    |
| SCCmec type IVb | GTCTCTTGTCAAGAAGGTTG | GTCTCTTGTCAAGAAGGTTG | 5 min, 94 °C; 30 cycles of 1 min, 94 °C; 1 min 51 °C; 2 min, 72 °C; final extension, 2 min, 72 °C | 493 [6]    |
| SCCmec type IVc | GTCTCTTGTCAAGAAGGTTG | GTCTCTTGTCAAGAAGGTTG | 5 min, 94 °C; 30 cycles of 1 min, 94 °C; 1 min 51 °C; 2 min, 72 °C; final extension, 2 min, 72 °C | 200 [6]    |
| SCCmec type IVd | GTCTCTTGTCAAGAAGGTTG | GTCTCTTGTCAAGAAGGTTG | 5 min, 94 °C; 30 cycles of 1 min, 94 °C; 1 min 51 °C; 2 min, 72 °C; final extension, 2 min, 72 °C | 881 [6]    |
| SCCmec type V | GAACATTGTTAATTAAATAGGCC/ | GAAAATTGTTAATTAAATAGGCC | 5 min, 94 °C; 30 cycles of 1 min, 94 °C; 1 min 51 °C; 2 min, 72 °C; final extension, 2 min, 72 °C | 325 [6]    |

**crr** type I

| Target       | Forward 5 → 3/ | Reverse 5 → 3  | PCR condition Expected size (bp) | Reference |
|--------------|----------------|----------------|----------------------------------|-----------|
| β: ATGCCTTTGAAATAGGCTT/ | a1: AACTTATCATGATACGATTG | ATGCCTTTGAAATAGGCTT/ | Multiplex PCR: 2 min, 94 °C; 30 cycles of 2 min, 94 °C; 1 min 57 °C; 2 min, 72 °C; final extension, 2 min, 72 °C | 937 [7]    |
| β: ATGCCTTTGAAATAGGCTT/ | a2: ATGCCTTTGAAATAGGCTT/ | ATGCCTTTGAAATAGGCTT/ | Multiplex PCR: 2 min, 94 °C; 30 cycles of 2 min, 94 °C; 1 min 57 °C; 2 min, 72 °C; final extension, 2 min, 72 °C | 1791 [7]   |
| β: ATGCCTTTGAAATAGGCTT/ | a3: ATGCCTTTGAAATAGGCTT/ | ATGCCTTTGAAATAGGCTT/ | Multiplex PCR: 2 min, 94 °C; 30 cycles of 2 min, 94 °C; 1 min 57 °C; 2 min, 72 °C; final extension, 2 min, 72 °C | 1287 [7]   |
| β: ATGCCTTTGAAATAGGCTT/ | a4: ATGCCTTTGAAATAGGCTT/ | ATGCCTTTGAAATAGGCTT/ | Multiplex PCR: 2 min, 94 °C; 30 cycles of 2 min, 94 °C; 1 min 57 °C; 2 min, 72 °C; final extension, 2 min, 72 °C | 518 [7]    |

**mec complex class A**

| Target       | Forward 5 → 3/ | Reverse 5 → 3  | PCR condition Expected size (bp) | Reference |
|--------------|----------------|----------------|----------------------------------|-----------|
| m7: ATATACCCAACCGACCAACTACA/ | IS4: GGATATGGCTGTCATGC | ATATACCCAACCGACCAACTACA/ | 2 min, 94 °C; 30 cycles of 2 min, 94 °C; 1 min 57 °C; 2 min, 72 °C; final extension, 2 min, 72 °C | 1963 [7]   |
| m6: CATATACCCAACCGACCAACTACA/ | ATATACCCAACCGACCAACTACA/ | ATATACCCAACCGACCAACTACA/ | 2 min, 94 °C; 30 cycles of 2 min, 94 °C; 1 min 57 °C; 2 min, 72 °C; final extension, 2 min, 72 °C | 1867 [3]   |
| m5: CATATACCCAACCGACCAACTACA/ | ATATACCCAACCGACCAACTACA/ | ATATACCCAACCGACCAACTACA/ | 2 min, 94 °C; 30 cycles of 2 min, 94 °C; 1 min 57 °C; 2 min, 72 °C; final extension, 2 min, 72 °C | 381 [3]    |
| m4: CATATACCCAACCGACCAACTACA/ | ATATACCCAACCGACCAACTACA/ | ATATACCCAACCGACCAACTACA/ | 2 min, 94 °C; 30 cycles of 2 min, 94 °C; 1 min 57 °C; 2 min, 72 °C; final extension, 2 min, 72 °C | 804 [7]    |

not only to methicillin, but also against other antibiotics because most resistance genes found in SCCmec have been also observed in coagulase-negative *Staphylococcus*). This study was performed to determine different types of SCCmec in *Staphylococcus epidermidis* (*S. epidermidis*) clinical isolates from intensive care unit (ICU) patients, Tehran, Iran.
mcc type C2 and undefined ccr type, and eight isolates had not amplified any gene with primers used here.

4. Discussion

Presence of *S. epidermidis* as a human flora causes this bacterium to be a usual carrier and reservoir for resistance genes[3]. The mecA gene encodes a penicillin-binding protein that is responsible for high-level resistance to methicillin in *S. epidermidis*. Recent data indicate that not only mecA but also other mobile genetic elements can be transferred from *S. epidermidis* to *S. aureus*[6]. Presence of mecA-positive *S. epidermidis* in the ICU and its transmission to patients can cause persistent infection which is difficult to treat. Resistant strains, particularly multidrug resistant ones will complicate the situation and cause serious problems in the treatment. Evidence suggests that SCCmec is able to transmit from *S. epidermidis* to *S. aureus* and can act as a reservoir and carrier for resistance genes by transferring horizontal gene[3]. From a total of 54 clinical isolates of mecA-positive *S. epidermidis*, three isolates (5.6%) possessed SCCmec type I, which did not show significant difference and is similar with previous reports of Garza-González et al.[8], Li et al.[9] and Bouchami et al.[10] in which, type I was not observed in *S. epidermidis*, but Mombach Pinheiro Machado et al. reported a rate of 28.7%[11], which is significantly higher than that of the present study. Also two isolates (3.7%) possessed SCCmec type II. This has been reported by Garza-González et al.[8], Mombach Pinheiro Machado et al.[11], Li et al.[9] and by Bouchami et al. (5.8%)[10], which has no significant difference with the present study. Li et al. reported that the value is 23.4%[12], which is significantly higher than that of present study. From a total of 54 clinical isolates of mecA-positive *S. epidermidis*, 29 isolates (53.7%) possessed SCCmec type III. This value has been reported by Garza-González et al. (30.8%)[8], by Mombach Pinheiro Machado et al. (27.6%)[11], by Li et al. (40.3%)[9] and by Bouchami et al. (32.4%)[10], which is significantly lower than that in the present study. Also three isolates (5.6%) possessed SCCmec type IV. The frequency of this type has been reported by Garza-González et al.[8], Li et al.[9] and Mombach Pinheiro Machado et al.[11], 3.8%, 1.2% and 3.9%, respectively, which is not significantly different with the present study. The frequency of type IV has been reported by Bouchami et al. (41.2%)[10], which is significantly higher than that of present study. Also, four isolates (7.4%) possessed SCCmec type V. This value has been reported by Garza-González et al. (3.8%)[8] and by Bouchami et al. (3%)[10], which is not significantly different from the findings of this study. Frequency of SCCmec type V has been reported by Li et al. (32.4%)[9], which is significantly more than that of present study. In mecA positive isolates, two isolates (3.7%) possessed SCCmec type VII. None of the previous studies reported this type. From the total of 54 mecA-positive isolates, 11 isolates (20.4%) could not show any type of SCCmec with primers of this study. This value has been reported by Garza-González et al. (15.4%)[8] and by Bouchami et al. (17.6%)[10], which is not significantly different from the findings of this study. About 3.4% has been reported by Mombach Pinheiro Machado et al.[11], which is significantly lower than that of this study. From a total of 11 nonclassified isolates, one isolate possessed mec type A and ccr type was unknown. One isolate possessed mec type B and ccr type was unknown. One isolate possessed mec type C2 and ccr type was unknown and 8 isolates did not develop any bonds with the primers used. In this study, the most prevalent SCCmec type was type III. For the isolates of study, two cases of SCCmec type VII were observed, but none has been reported in other similar studies. The SCCmec type III was the predominant SCCmec type in the present study which is significantly higher than similar studies. Eleven of 54 SCCmec types were not classified, among which one isolate possessed mec type A and ccr type was unknown. Furthermore, the SCCmec type VII was amplified in 3.7% of isolates, and this SCCmec type was reported in *S. epidermidis* for the first time.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

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