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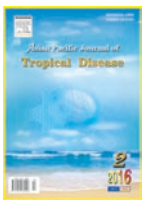

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Diversity of the SCC*mec* types among *Staphylococcus epidermidis* clinical isolates from intensive care unit patients

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ABSTRACT

Objective: To determine different types of staphylococcal cassette chromosome *mec* (SCC*mec*) 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 SCC*mec* type was the SCC*mec* type III which was detected in 53.7% of isolates. The frequencies of other SCC*mec* types were as following: 5.6% in SCC*mec* type I, 3.7% in SCC*mec* type II, 5.6% in SCC*mec* type IV, 7.4% in SCC*mec* type V and 3.7% in SCC*mec* type VII. Furthermore, 20.3% of tested isolates were not typeable using the specific primers.

Conclusions: SCC*mec* type III was the most frequent SCC*mec* type in this study. Eleven of 54 SCC*mec* 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 SCC*mec* 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* (SCC*mec*) elements. The origin of SCC*mec* 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 SCC*mec* 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 SCC*mec* types have been identified in *S. aureus*, and coded as I-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 *Staphylococci* 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* SCC*mec* types exhibit resistance

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not only to methicillin, but also against other antibiotics because most resistance genes found in SCC*mec* have been also observed in coagulase-negative *Staphylococci*[3]. This study was performed to determine different types of SCC*mec* in *Staphylococcus epidermidis* (*S. epidermidis*) clinical isolates from intensive care unit (ICU) patients, Tehran, Iran.

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 on sheep blood agar, tube coagulase, catalase, carbohydrate fermentation 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 ℃ for 5 min, then 0 ℃ 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 SCC*mec*, the presence of *mec*A was investigated in all isolates, then SCC*mec* 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 SCC*mec* type was SCC*mec* type III which was observed in 53.7% (95% *CI*: 40.4–67.0) of isolates. Frequencies of other SCC*mec* types were as following: 5.6% (95% *CI*: 0.0–11.7) in SCC*mec* 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

Primers and PCR condition for *mecA*, SCC*mec* type I-V, *ccr* type and *mec* complex identification.

Target	Forward 5→3/ Reverse 5→3	PCR condition	Expected size (bp)	Reference
mecA	GTGAAGATATACCAAGTGAAT/ ATGCGCTATAGATTGAAAGGAT	4 min, 94 ℃; 35 cycles of 1 min, 94 ℃; 1 min 57 ℃; 1 min, 72 ℃; final extension, 10 min, 72 ℃	147	[6]
SCC*mec* type I	GCTTTAAAGAGTGTCGTTACAGG/ GTCTCTCATAGTATGACGTCC	Multiplex PCR: 5 min, 94 ℃; 30 cycles of 1 min, 94 ℃; 1 min, 51 ℃; 1 min, 72 ℃; final extension, 10 min, 72 ℃	613	[6]
SCC*mec* type II	CGTTGAAGATGATGAAGCG/ CGAAATCAATGGTTAATGGACC		398	[6]
SCC*mec* type III	CCATATTGTGTACGATGCG/ CCTTAGTTGTCGTAACAGATCG		280	[6]
SCCmec type IVa	GCCTTATTCGAAGAAACCG/ CTACTCTTCTGAAAAGCGTCG		776	[6]
SCC*mec* type IVb	TCTGGAATTACTTCAGCTGC/ AAACAATATTGCTCTCCCTC		493	[6]
SCC*mec* type IVc	ACATATTTGTATTATCGGAGAGC/ TTGGTATGAGGTATTGCTGG		200	[6]
SCC*mec* type IVd	CTCAAAATACGGACCCCAATACA/ TGCTCCAGTAATTGCTAAAG		881	[6]
SCC*mec* type V	GAACATTGTTACTTAAATGAGCG/ TGAAAGTTGTACCCTTGACACC		325	[6]
ccr type I	βc: ATTGCCTTGATAATAGCCITCT/ α1: AACCTATATCATCAATCAGTACGT	Multiplex PCR: 2 min, 94 ℃; 30 cycles of 2 min, 94 ℃; 1 min, 57 ℃; 2 min, 72 ℃; final extension, 2 min, 72 ℃	695	[7]
ccr type II	βc: ATTGCCTTGATAATAGCCITCT/ α2: TAAAGGCATCAATGCACAAACACT		937	[7]
ccr type III	βc: ATTGCCTTGATAATAGCCITCT/ α3: AGCTCAAAAGCAAGCAATAGAAT		1 791	[7]
ccr type IV	α4.2: GTATCAATGCACCAGAACTT/ β4.2: TTGCGACTCTCTTGGCGTTT		1 287	[7]
ccr type V	γF: CGTCTATTACAAGATGTTAAGGATAAT/ γR: CCTTTATAGACTGGATTATTCAAAATAT		518	[7]
mec comlex class A	mA7: ATATACCAAACCCGACAACTACA/ mI6: CATAACTTCCCATTCTGCAGATG	2 min, 94 ℃; 30 cycles of 2 min, 94 ℃; 1 min, 60 ℃; 2 min, 72 ℃; final extension, 2 min, 72 ℃	1 963	[7]
mec comlex class B	mA7: ATATACCAAACCCGACAACTACA/ mDA2: GATGTCTGTCGAGGACTC	2 min, 94 ℃; 30 cycles of 2 min, 94 ℃; 1 min, 60 ℃; 2 min, 72 ℃; final extension, 2 min, 72 ℃	1 687	[3]
mec comlex class C1	mA7: ATATACCAAACCCGACAACTACA/ ISF4: CGGATTTTCGCCATGCCACGA	2 min, 94 ℃; 30 cycles of 2 min, 94 ℃; 1 min, 60 ℃; 2 min, 72 ℃; final extension, 2 min, 72 ℃	381	[3]
mec comlex class C2	mA7: ATATACCAAACCCGACAACTACA IS2(iS-2):TGAGGTTATTCAGATATTTCGATGT	2 min, 94 ℃; 30 cycles of 2 min, 94 ℃; 1 min, 60 ℃; 2 min, 72 ℃; final extension, 2 min, 72 ℃	804	[7]

mec 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 SCC*mec* is able to transmit from *S. epidermidis* to *S. aureus* and can act as a reservoir and carrier for resistance genes by transfering horizontal gene[3]. From a total of 54 clinical isolates of *mecA*-positive *S. epidermidis*, three isolates (5.6%) possessed SCC*mec* 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 in the present study. Also two isolates (3.7%) possessed SCC*mec* type II. This value has been reported by Garza-González *et al*.[8], Mombach Pinheiro Machado *et al*.[11], Li *et al.* (0%)[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 SCC*mec* 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 SCC*mec* 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 SCC*mec* 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 SCC*mec* 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 SCC*mec* 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 SCC*mec* 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 SCC*mec* type was type III. For the isolates of study, two cases of SCC*mec* type VII were observed, but none has been reported in other similar studies. The SCC*mec* type III was the predominant SCC*mec* type in the present study which is significantly higher than similar studies. Eleven of 54 SCC*mec* types were not classified, among which one isolate possessed *mec* type A and *ccr* type was unknown. Furthermore, the SCC*mec* type VII was amplified in 3.7% of isolates, and this SCC*mec* 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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