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Molecular Characterization of *Staphylococcus epidermidis* Isolates Collected From an Intensive Care Unit

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Abstract

Background: *Staphylococcus epidermidis* is known as the most significant cause of nosocomial infections. Moreover, bloodstream infection is one of the noticeable and common infections in many wards of health care units, especially in intensive care units (ICU) and neonatal intensive care units (NICU). It has been proved that the *mecA* gene is the principal cause of methicillin resistance in *Staphylococcus epidermidis* strains. Also, *mecA* and other genes are located on *staphylococcal cassette* chromosome mec (SCCmec).
Objectives: The aim of this study was investigating the genotypic characteristics of methicillin resistant *Staphylococcus epidermidis* (MRSE) strains isolated from hospitalized patients at the intensive care unit.
Methods: A total of 121 isolates were recovered from bloodstream infections of ICU hospitalized patients in Al-Zahra hospital (Isfahan, Iran). Overall, fifty-three isolates belonged to *S. epidermidis*. Antibiotic susceptibility Test, determination of *mecA* gene, SCCmec types, Pulse Field Gel Electrophoresis (PFGE) and Multi-Locus Sequence Typing (MLST) methods were carried out as the preferential techniques.
Results: In accordance to our study, 43.4% of isolates were resistant to cefoxitin, while the *mecA* gene was found in 25 (47.2%) isolates by the PCR method. In addition, various SCCmec types were detected from MRSE strains by using multiplex polymerase chain reaction (PCR). Furthermore, a total of 16 different pulsotypes were identified with PFGE typing via GelCompar II analysis. It is notable that the most prevalent ST type was ST2.
Conclusions: Recognizing the source of infection is essential for monitoring the dissemination of infections, therefore this important issue is not acquired without various typing methods.

Keywords: Molecular Characterization, PCR, ICU, *S. epidermidis*

1. Background

Staphylococcus epidermidis is one of the main members of coagulase-negative staphylococci (CoNS), and may cause severe infections, such as bloodstream, medical implant devices, peripheral or central intravenous catheters (CVCs), Eye keratitis, and nosocomial infections (1, 2). Bloodstream infection is one of the most noticeable and common infections in many wards of health care units, especially in the intensive care unit (ICU) and neonatal intensive care unit (NICU) (3). The existence of virulence factors (biofilm formation, polysaccharide intercellular adhesion, phenol-soluble modulin and antibiotic resistance genes) and moreover the prevalence of resistant strains to many antimicrobial agents such as beta-lactams are important reasons of speared of infections in coagulase-negative related diseases. In the two past decades the incidence of Methicillin-resistant *Staphylococcus epidermidis* (MRSE) strains is becoming a global concern in ICU and other wards of hospitals (4, 5). The presence of the *mecA* gene is the main cause of MRSE emergence, and is related to a penicillin binding protein (PBP2a). The *mecA*, *mecI* and *mecR1* are important elements of the mec operon, which are located on Staphylococcal Cassette Chromosome mec (SCCmec) (6). Up to now different types of SCCmec elements have been identified, however type I to V are most commonly found in *Staphylococcus epidermidis* strains (7). Since the SCCmec elements are classified on the basis of their various structural attributes, this kind of typing is considered a powerful method for MRSE epidemiological studies (8). On the other hand, Pulse Field Gel Electrophoresis (PFGE) is regarded as one of the gold standard methods for *S. epidermidis* epidemiological studies and is also useful for tracking specific outbreaks (9). It should be noted that the Multi-Locus Sequence Typing (MLST) method is an-

other prominent typing technique for investigating many bacterial pathogens, which lead to nosocomial infections, including *S. epidermidis* (10).

2. Objectives

The aim of this study was to evaluate the molecular characterization of MRSE strains isolated from hospitalized patients in an ICU ward with SCCmec, PFGE and MLST typing methods.

3. Methods

3.1. Bacterial Isolates and Identification

In the six-month period of the study, 121 isolates were recovered from bloodstream infections of ICU hospitalized patients in Al-Zahra hospital (Isfahan, Iran). Among the collected isolates, 53 isolates were identified as *Staphylococcus epidermidis* and other isolates were *Escherichia coli* and *Pseudomonas aeruginosa*, after identification with primary microbiological tests (2). *Staphylococcus epidermidis* isolates were transferred to the microbiology laboratory and confirmed with microbiological standard tests such as Gram staining, catalase, coagulase activity testing with rabbit plasma, DNase, mannitol salt agar, and thereafter stored at -20°C for molecular supplementary tests.

3.2. Antimicrobial Resistance Profiles

Antibiogram was verified by disc diffusion method on Mueller-Hinton agar, according to Clinical and Laboratory Standards Institute (CLSI) guidelines (11). The antimicrobial agents included cefoxitin (30 μg), penicillin (10 IU), cefazolin (30 μg), vancomycin (30 μg), erythromycin (15 μg), gentamicin (10 μg), sulfamethoxazole (23.75 μg) + trimethoprim (1.25 μg), rifampicin (5 μg), levofloxacin (5 μg), clindamycin (2 μg) and teicoplanin (30 μg).

3.3. DNA Extraction

DNA extraction was performed using a DNA extraction kit (Qiagene, United States), according to the manufacturer's protocol. It should be mentioned that 1.5 μL of lysostaphin was added to the final bacterial suspension (12).

3.4. Determination of mecA Gene and SCCmec Types

Detection of *mecA* gene and various SCCmec types (I-V) was carried out by the multiplex polymerase chain reaction (PCR) approach with subsequent visualization of the amplified DNA fragment patterns by 1% agarose gel electrophoresis and SYBR® Safe DNA gel stain (Invitrogen, USA). Specific primers and PCR conditions were completed as described previously (12).

3.5. Pulsed Field Gel Electrophoresis

Methicillin-resistant *Staphylococcus epidermidis* strains were genotyped by Pulsed field gel electrophoresis (PFGE) using the restriction enzyme *SmaI* (Takara, Japan) and conducted according to methods described previously (13). Macrorestriction profiles were analyzed by GelCompar II version 4 (Applied Maths, Sint-Martens-Latem, Bel-gium) with 90% similarity cutoff point and clustered by unweighted pair group method with arithmetic mean (UPGMA).

3.6. Multi-Locus Sequence Typing

Polymerase chain reaction of the seven housekeeping genes encoding carbamate kinase (*arcC*), shikimate dehydrogenase (*aroE*), ABC transporter (*gtr*), DNA mismatch repair protein (mutS), pyrimidine operon regulatory protein (pyirR), triosephosphate isomerase (*tpiA*) and acetyl-CoA acetyltransferase (*yqiL*) was performed as explained previously (14). The allelic profiles and also sequence types were analysis with (http://www.mlst.net).

4. Results

During six months of the study, 121 bloodstream infections were collected from the ICU ward of Al-Zahra hospital. Fifty-three of all isolates belonged to *S. epidermidis*. The other covered isolates were associated with *E. coli* and *P. aeruginosa*. Since many factors were regarded for the determination of the clinical significance of coagulase negative staphylococcus diseases, in which the most prominent was center for disease control (CDC) standards, therefore all of the *S. epidermidis* isolates were gathered according to the mentioned guidelines (15). The antibiogram showed the following results: cefoxitin (43.4%), penicillin (100%), cefazolin (94.3%), vancomycin (0%), erythromycin (77.3%), gentamicin (73.6%), sulfamethoxazole/trimethoprim (56.5%), rifampicin (39.6%), levofloxacin (52.8%), clindamycin (43.4%) and teicoplanin (0%).

In the present study, 25 (47.2%) of *S. epidermidis* strains had the *mecA* gene. In addition, by multiplex PCR, SCCmec type II was detected in one (4%), SCCmec type III in eight (32%) and SCCmec type IV in thirteen (52%) of isolates. It is noteworthy to mention that three (12%) of the isolates were distinguished as non-typable and also SCCmec types I and V were not detected.

Using PFGE typing by GelCompar II analysis, a total of 16 different pulsotypes were identified, including four main clones (I-IV). In PFGE dendrogram clone I with two, clone II with three, clone III with two and clone IV with six strains had the most frequency (Figure 1).

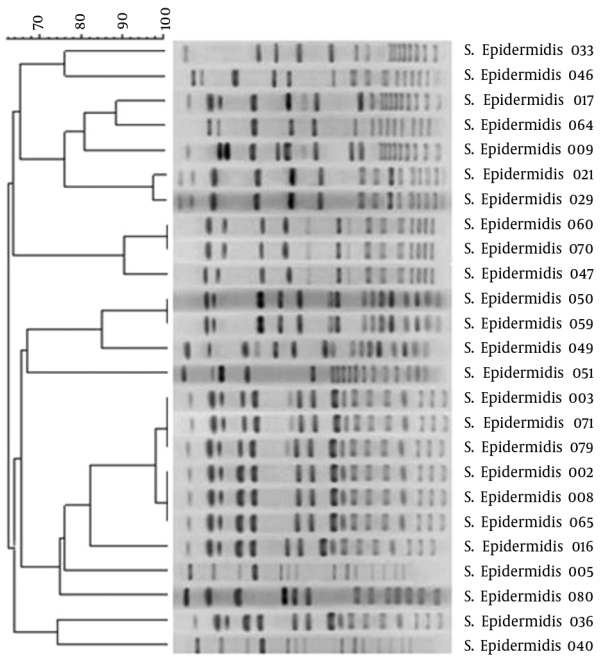

Figure 1. Pulsed-Field Gel Electrophoresis Dendrogram of Twenty-five Methicillin-resistant *Staphylococcus epidermidis* Isolates Recovered From the Intensive Care Unit ward of Al-Zahra Hospital, with a 90% Similarity Cut-Off Point Clustered by Unweighted Pair Group Method with Arithmetic Mean

Finally the MLST was carried out for I-IV detected clones by PFGE profiles. From clone I (strains 21 and 29), clone II (strains 47 and 60), clone III (strain 50 and 59) and clone IV (strains 3 and 8) were selected for MLST test, in accordance to SCCmec types. The MLST results are shown in Table 1.

Table 1. Various Sequence Types were Detected in Methicillin-resistant *Staphylococcus epidermidis* Isolates Collected From the Intensive Care Unit Under Study

Sequence Type	No. (%)
ST2	5 (62.5)
ST5	2 (25)
ST27	1 (12.5)

5. Discussion

According to many studies, nosocomial dissemination of *S. epidermidis* strains and the genetic diversity due to horizontal gene transferring and mobile genetic elements and exchanging may lead to different infections in various wards of health care units such as ICU (16). Currently researchers are aware that *S. epidermidis* and other (CoNS) are a reservoir of SCCmec elements and antimicrobial resistance genes.

The purpose of this study was to determine the SCCmec elements, PFGE pattern and the MLST of MRSE strains isolated from ICU wards. Antibiotic susceptibility testing results showed multidrug resistant isolates.

In the present study, the high antimicrobial resistance rates were evaluated in MRSE strains and 75.5% of the isolates were resistant to more than four antibiotics. In PCR molecular test, 47.2% of strains were distinguished as MRSE. It is important to note that no vancomycin and teicoplanin resistance were observed in our studied strains. Rifampicin with 39.6% resistance, had the lowest resistance among of antimicrobial tested agents.

In the study of Al Tayyar et al. 92 (41.2%) of the clinical specimens were isolated from the ICU ward. Of these, 69 (30.9%) of the specimens were associated with bloodstream infections. Furthermore, 54.7% of isolates belonged to *S. epidermidis* strains. In antimicrobial susceptibility test, vancomycin, linezolid, rifampin and nitrofurantoin showed high sensitivity while, the lowest sensitivity rate was distinguished in ampicillin, penicillin, ceftriaxone, cefazolin, amoxicillin-clavulanic acid and erythromycin (17).

In a similar study, which was done in Indonesia at the ICU ward, the main isolates were *P. aeruginosa*, *K. pneumoniae* and *S. epidermidis*. High rate of resistance to cephalexin and also levofloxacin was detected among *S. epidermidis* isolates (18).

In SCCmec typing, the prevalence of type IV of SCCmec was notable. These results were similar to Wisplinghoff et al. and other related studies (19). However in comparison to related studies about *S. aureus* isolates, the prevalence of SCCmec type III had the highest percentage (20, 21).

By the PFGE method, with 16 different pulsotypes, four main clones were obtained, amongst which, clone IV with six strains was the most common. This result showed the wide diversity of isolates by PFGE typing method. According to Table 2, most of the SCCmec results and PFGE patterns were coordinated with each others, for example, clone I or (F) had the same SCCmec type (IV) or clone IV or (K).

Multi-Locus Sequence Typing was performed on eight isolates, wherein three STs, including ST2, ST5 and ST27, were distinguished. ST2 showed the highest ratio in our studied isolates. From clone I (F1 and F2) each of the two strains were examined by MLST technique, while both strains had SCCmec type IV but the STs were different from each other (ST2 and ST5). From clone II (G1, G1 and G2) two strains (47 and 60) with the same STs were detected, while the SCCmec types were various. In clone III (H1 and H1) both strains (50 and 59) with different SCCmec types had the identical STs (ST2) and in clone IV with six strains, both

Table 2. Methicillin-Resistant *Staphylococcus epidermidis* Isolates and Corresponding SCCmec Types, Pulse Field Gel Electrophoresis Profiles and STs

Strains	Gender	Age	*mecA* gene	SCCmec Type	PFGE Profile	Allelic Profile	ST
2	M	52	Positive	Non-Typable	K2	-	-
3	M	63	Positive	IV	K1	1-1-1-2-2-1-1	ST5
5	F	45	Positive	IV	M	-	-
8	M	64	Positive	IV	K2	1-2-2-2-2-3-1	ST27
9	M	69	Positive	III	E	-	-
16	M	53	Positive	IV	C	-	-
17	F	50	Positive	II	L	-	-
21	F	49	Positive	IV	F1	7-1-2-2-4-1-1	ST2
29	M	18	Positive	IV	F2	1-1-1-2-2-1-1	ST5
33	M	68	Positive	III	A	-	-
36	F	49	Positive	IV	O	-	-
40	M	55	Positive	III	P	-	-
46	F	37	Positive	IV	B	-	-
47	M	77	Positive	IV	G2	7-1-2-2-4-1-1	ST2
49	M	49	Positive	IV	I	-	-
50	M	56	Positive	III	H1	7-1-2-2-4-1-1	ST2
51	F	68	Positive	Non-Typable	J	-	-
59	M	70	Positive	IV	H1	7-1-2-2-4-1-1	ST2
60	M	38	Positive	III	G1	7-1-2-2-4-1-1	ST2
64	F	67	Positive	III	D	-	-
65	M	53	Positive	IV	K2	-	-
70	M	71	Positive	III	G1	-	-
71	F	66	Positive	IV	K1	-	-
79	F	76	Positive	Non-Typable	K1	-	-
80	M	48	Positive	III	N	-	-

strains number 3 (ST5) and 8 (ST27), were evaluated with MLST. In a related study, which was performed on blood cultures in Germany, 13 different STs were determined. The most common STs were allocated to sequence types ST2, ST5, ST10 and ST242. In our study, ST2 and ST5 had the highest prevalence sequence types, similar to the present study (22).

According to our research, the SCCmec typing, PFGE and MLST results had conformity with each other. To compare SCCmec and PFGE with established typing methods, we also analyzed eight of the *S. epidermidis* isolates by MLST. Taken alone within the studied *S. epidermidis* isolates, the PFGE data suggest a high degree of genotypic diversity; nevertheless these results have been verified by other investigations (23).

These great variations, which were observed using PFGE, suggest genetic diversity of various strains isolated from different patients, but in MLST a measure of genetic changes for housekeeping genes was involved in our targets so the rate of genetic diversity was happened slowly (24). This investigation indicated that from 53 *S. epidermidis* isolates, 47.2% were MRSE, which led to nosocomial infections in the ICU. On the other hand, the *S. epidermidis* with various SCCmec elements and resistance genes may lead to severe infections in health care units and accelerate nosocomial infections. The emergence of drug resistance amongst *S. epidermidis* strains is very critical point for nosocomial infections, especially for ICU and NICU patients, hence recognizing the source of infection is very essential to monitor the dissemination of infections, therefore this important issue is not addressed without various typing methods. According to antibiotic susceptibility test and molecular typing results, we suggest that it is better to evaluate important virulence factors of this microorgan-

ism because there may be a correlation between these results and virulent strains.

Footnote

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