DOI: 10.7860/JCDR/2017/25518.10258

Original Article

Microbiology Section

# Distribution of *SCCmec* Elements and Presence of Panton-Valentine Leukocidin in Methicillin-Resistant *Staphylococcus epidermidis* Isolated from Clinical Samples in a University Hospital of Isfahan City, Iran

MEHRDAD HALAJI[1], ASHKAN KARIMI[2], PARISA SHOAEI[3], MOHAMMADREZA NAHAEI[4], FARZIN KHORVASH[5], BEHROOZ ATAEI[6], MAJID YARAN[7], SEYED ASGHAR HAVAEI[8]

## ABSTRACT

**Introduction:** Coagulase Negative *Staphylococcus* (CoNS) is considered as a major pathogen of nosocomial infections among immunosuppressed patients.

**Aim:** The aim of this study was to identify the types of *Staphylococcal Cassette Chromosome mec* (*SCCmec*) and Panton-Valentine Leukocidin (*PVL*) gene among clinical Methicillin-Resistant *S. epidermidis (MRSE)* isolates collected from Isfahan.

**Materials and Methods:** This cross-sectional study was performed from March 2014 to January 2015 at a tertiary care hospital of Isfahan, Iran. Antimicrobial susceptibility tests of *S. epidermidis* isolates were performed by the disc diffusion method. All the strains were screened for methicillin resistance based on resistance to cefoxitin (30 µg) disc and presence of *mecA* gene. Determination of *SCCmec* typing and *PVL* toxin gene were performed by PCR method. For categorical variables different groups were compared using the Chi-square test or Fisher exact test. A p-value of <0.05 was considered significant for all statistical tests.

**Results:** The frequency of MRSE was 53.8% according to the presence of *mecA* gene. The overall resistance rate was high with ciprofloxacin (81.4%). PCR analysis showed that 17% (12/70) of MRSE isolate carried the PVL gene and 43% (30/70) were *SCCmec* type I; 11.4% (8/70) were type II; and 34.2% (24/70) were type IV, whereas, 11.4% (8/70) of the MRSE isolates could not be typed.

**Conclusion:** *SCCmec* type I was the major type of *SCCmec*, which indicates an emergence of this *SCCmec* type in the studied medical centers. Increased prevalence of *SCCmec* types in community is cause of an increase in antibiotic resistance among microorganisms.

**Keywords:** Antibiotic resistance, Cefoxitin, Chromosome, Cytotoxins

## INTRODUCTION

Coagulase-Negative Staphylococcus (CoNS) is considered as an important agent of nosocomial or healthcare-associated infections among immunosuppressed patients and elderly individuals [1,2]. In the past decades, CoNS was considered as a commensal bacterium and normal bacterial flora of healthy human skin, but currently, these organisms are considered as common cause of human diseases [3]. CoNS has also been found the third most common causative agent of nosocomial infections. Among CoNS, *Staphylococcus epidermidis* is an important pathogen in neonatal sepsis and patients who use medical equipments such as urinary catheters and other implanted devices [4]. Approximately 75%-90% of these bacteria, circulating in hospitals are resistance to Methicillin [5-7]. The resistance of *S. epidermidis* to methicillin is encoded by the expression of Penicillin Binding Protein (PBP2a) with a low affinity for this antibiotic. PBP2a is encoded by the *mecA* gene, which is located on a mobile genetic element and it is called the *SCCmec* [8].

*SCCmec* is widely disseminated among *Staphylococcus aureus* and CoNS species. Generally, *SCCmec* types are defined based on mec gene complex and ccr (Cassette Chromosome Recombinases) gene complex [9,10]. In addition, *SCCmec* are divided into subtype according to difference in the j region DNA. According to previous reports, in *S. aureus* 11 types of *SCCmec* and several subtypes have been observed [11,12]. In comparison with *S. aureus*, molecular characterization of this element revealed five *SCCmec* types in *S. epidermidis* [2,13]. Due to the presence of new variants of ccr genes, *SCCmec* structures are more diverse in *S. epidermidis*. Therefore, these results indicate a high percentage of genetic diversity within the *SCCmec* elements carried by *S. epidermidis* [14,15]. So far, several molecular typing techniques including Pulsed Field Gel Electrophoresis (PFGE), Multilocus Sequence Typing (MLST) and *SCCmec* typing have been used for investigating the spread of MRSE. These molecular typing techniques enable enhancing clinical therapy and infection control in the future by presenting useful data. *SCCmec* types have been used to distinguish Hospital-Associated Methicillin-Resistant *Staphylococcus* (HA-MRS) from Community-Associated Methicillin Resistant *Staphylococcus* (CA-MRS) [16]. Some *S. epidermidis* strains produce PVL cytotoxin and biofilm as key virulence factors which increase the pathogenecity of these microorganisms. PVL is a cytotoxin that causes damage to the membranes of host defense cells and is associated with tissue necrosis as well [17-19]. Since, there is no adequate data about the prevalence of *SCCmec* diversity and *PVL* gene among clinical isolates of *S. epidermidis* in our region, the present study aimed to identify the types of *SCCmec* and *PVL* gene among clinical MRSE isolates collected from Isfahan.

## MATERIALS AND METHODS

### Study Design and Population

This cross-sectional study was carried out from March 2014 to January 2015 in Al-Zahra hospital, affiliated to Isfahan University of Medical Sciences, Isfahan, Iran. All the parts of study were supervised and approved by the Ethical Committee of Isfahan University of Medical Sciences (code 293253).

### Bacterial Isolation and Identification

One hundred and thirty *S. epidermidis* isolates were collected from clinical samples of patients who referred to Isfahan's AL-Zahra hospital (Iran). The isolates were recovered from wounds (n=30), blood (n=18), urine (n=60), sputum (n=7), Bronchoalveolar Lavage (BAL) (n=5), and abscesses (n=10). Thereafter, all isolates were identified as *S. epidermidis* using different conventional methods including Gram staining, catalase test, plasma coagulase test, and final identification of *S. epidermidis* was performed by the ability of urease production and carbohydrate fermentation [10]. All strains were stored at -70°C in Trypticase Soy Broth (TSB), with 20% glycerol.

### Antimicrobial Susceptibility Assay

Antimicrobial susceptibility tests of *S. epidermidis* isolates were performed by the disc diffusion method on Mueller– Hinton agar (Himedia, India) according to the guidelines of Clinical and Laboratory Standards Institute (CLSI) for gentamycin (10 µg), oxacillin (1 µg), tetracycline (30 µg), ciprofloxacin (5 µg), clindamycin (2 µg), Co-Trimoxazole (SXT- 1.25/23.75 µg), rifampin (5 µg), and vancomycin antibiotic discs (MAST, UK) [20]. *S. aureus* ATCC® 25923™, which is a methicillin-sensitive *S. aureus*, was used as the control strain in antibacterial susceptibility testing. All the strains were screened for methicillin resistance based on resistance to cefoxitin (30 µg) discs (MAST, UK) by the disc diffusion method according to the CLSI 2013 guidelines.

### DNA Extraction and PCR Assay

Bacterial genomic DNA of suspected isolates were extracted directly from 24 hours colonies by boiling a dense suspension in sterile distilled water for 10 minutes and centrifuged for two minutes at 13,000×g [21]. The genomic DNA was used as a template for typing of *SCCmec*, detection of *mecA* and the *PVL* cytotoxin gene. The PCR for amplification of *mecA* gene was performed as described previously [22].

### Detection of the *PVL* Gene

PCR was performed to determine the presence of the *PVL* cytotoxin gene as previously described by Azimian A et al., [22].

### *SCCmec* Typing by Multiplex-PCR

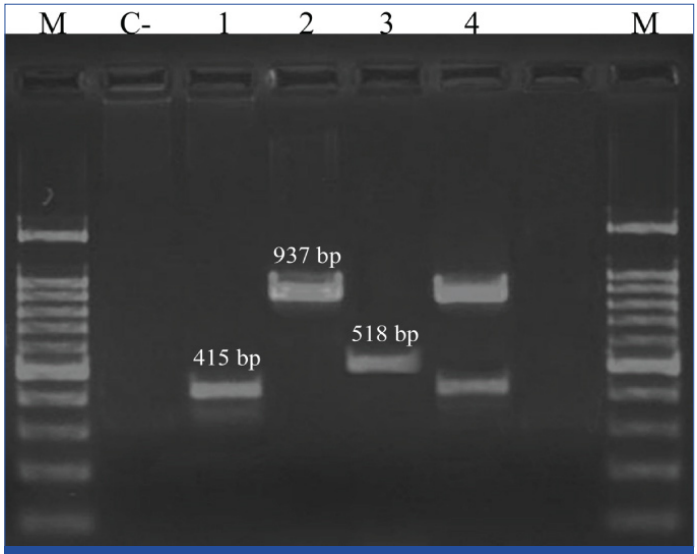
The *SCCmec* type was determined in a multiplex PCR which included four pairs of primers for the *SCCmec* types I- IV. PCR was performed in a mixture of 30 µl volume containing 15 µl master mix, 0.5 µl of each of α3R1, βF1, ccrCF and ccrCR primers (10 pmol/µl), 0.3 µl of each of 1272F1, 1272R1, 5RmecAF, 5R431R primers (10 pmol/µl) and 2 µl template DNA. The final volume was adjusted to 30 µl by adding 9.8 µl sterile ultrapure water and was placed on thermal cycler [23]. Amplification protocol consisted of five minutes initial denaturation at 93°C, followed by 30 cycles of denaturation (93°C/45 seconds), annealing (55°C/30 seconds), and extension (72°C/90 seconds), and an additional post-amplification extension step at 72°C for five minutes. The primers used for the PCR are listed in [Table/Fig-1]. The following *S. aureus* reference strains were used as controls: COL; N315; 85/2082; and JCSC/4469 for the *SCCmec* types I, II, III, and IV, respectively [Table/Fig-2].

## STATISTICAL ANALYSIS

Data were entered and analysed by Statistical Package for the Social Sciences (SPSS) software version 20.0. The description of the results was carried out by frequencies. For categorical variables different groups were compared using the Chi-square test or Fisher-exact test. All probabilities were two-tailed and a p-values < 0.05 was considered to be statistically significant.

| Target | Primer | Sequence | Product size (bp) | Reference |
|---|---|---|---|---|
| *SCCmec* | β F1 | ATTGCCTTGATAATAGCCYTCT | 937 | [23] |
| | α3 R1 | TAAAGGCATCAATGCACAAACACT | | |
| | ccrC F | CGTCTATTACAAGATGTTAAGGATAAT | 518 | [23] |
| | ccrC R | CCTTTATAGACTGGATTATTCAAAATAT | | |
| | 1272 F | GCCACTCATAACATATGGAA | 415 | [23] |
| | 1272 R | CATCCGAGTGAAACCCAAA | | |
| | 5RmecAF | TATACCAAACCCGACAACTAC | 359 | [23] |
| | 5R431R | CGGCTACAGTGATAACATCC | | |
| *PVL* | *PVL* F | GGAAACATTTATTCTGGCTATAC | 502 | [22] |
| | *PVL* R | CTGGATTGAAGTTACCTCTGG | | |
| *mecA* | F | AGAAGATGGTATGTGGAAGTTAG | 583 | [23] |
| | R | ATGTATGTGCGATTGTATTGC | | |

**[Table/Fig-1]:** List of primers used in this study.
*F: Forward, R: Reverse




**[Table/Fig-2]:** C-: negative control (distilled water); lane 1: *SCCmec* type I (COL); lane 2: *SCCmec* type II (N315); lane 3: *SCCmec* type 3 (85/2082); lane 4: *SCCmec* type IV (JCSC/4469); M: 100 bp ladder.

## RESULTS

### Frequency of MRSE

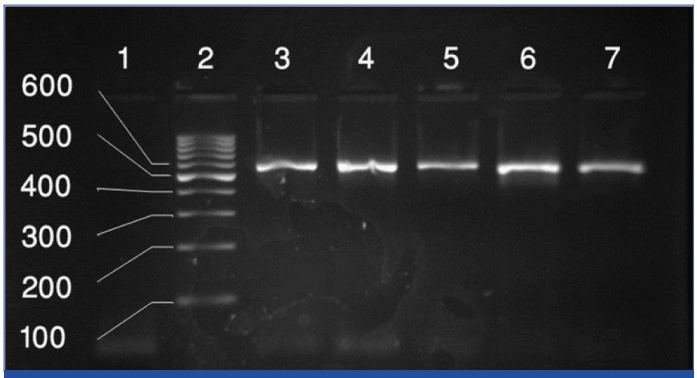
Out of total 130 *S. epidermidis* isolates, the frequency of MRSE was 52.3% (n=68) and 53.8% (n=70) according to the disc diffusion and presence of *mecA* gene, respectively. Phenotypic (Cefoxitin disc) and molecular (*mecA* detection) based MRSE screening methods showed different results [Table/Fig-3]. Specimen wise distribution showed that MRSE was most frequent in urine (53%), followed by blood (11.4%), sputum (5.7%), wound (25.6%) and BAL (4.3%). On the other hand, the frequency of MRSE was found to be 42.7% and 57.3% for female and male respectively.

### Antibiotic Susceptibility Pattern of *S. epidermidis* Isolates

The antibiotic susceptibility showed that all MRSE isolates were 100% sensitive to vancomycin. The overall resistance rates were high with ciprofloxacin (81.4%) and co-trimoxazole (64%), while the lowest level of antibiotics resistance were against gentamycin (37.1%). [Table/Fig-4] shows the distribution of antibiotic resistance among MRSE isolates based on *SCCmec* type.

### Characterization of *SCCmec* Type and *PVL* Gene

Results from PCR analysis shown that 12 (17%) of MRSE isolates carried the *PVL* gene [Table/Fig-5]. In addition, of the 70 MRSE isolates, 43% (30/70) were *SCCmec* type I; 11.4% (8/70) were

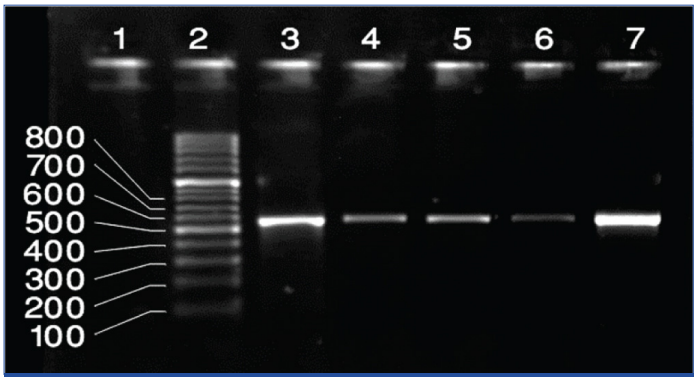



**[Table/Fig-3]:** PCR amplification of the *mecA* genes. Lane 1: Negative Control, Lane 2: 100 bp-3k b ladder, lane 3: positive control for *mecA* genes (583bp), lane 4-7: positive results for *mecA* genes.

| Antibiotic | *SCCmec* I (n=30) | *SCCmec* II (n=8) | *SCCmec* IV (n=24) | Untypeable (n=8) | Total (n=70) |
|---|---|---|---|---|---|
| Gentamycin | 11 | 1 | 12 | 2 | 26 |
| Oxacillin | 24 | 5 | 24 | 6 | 59 |
| Tetracycline | 23 | 3 | 15 | 3 | 44 |
| Ciprofloxacin | 26 | 6 | 19 | 6 | 57 |
| Clindamycin | 15 | 4 | 15 | 4 | 38 |
| Co-trimoxazole | 19 | 5 | 15 | 6 | 45 |
| Rifampin | 14 | 3 | 15 | 0 | 32 |
| Vancomycin | 0 | 0 | 0 | 0 | 0 |

**[Table/Fig-4]:** Distribution of antibiotic resistance among MRSE isolates by *SCCmec* types.

type II; 34.2% (24/70) were type IV and 11.4 (8/70) of the MRSE isolates could not be typed, whereas, none of the isolates were identified as *SCCmec* type III [Table/Fig-6]. The *SCCmec* type I of MRSE isolates were significantly ($p<0.05$) recovered as the major type from 19 (63.3%) urine followed by 5 (16.7%) from wound, 3 (10%) from blood and 3 (10%) from sputum, while the *SCCmec* type II isolates were 4 (50%) from sputum, 3 (37.5%) from urine, 1 (12.5%) from wound and the *SCCmec* type IV isolates were mostly 13 (54.1%) isolated from the urine samples, 8 (33.4%) from blood and 3 (12.5%) from sputum. Also, the majority of PVL-positive isolates were *SCCmec* type I (50%).

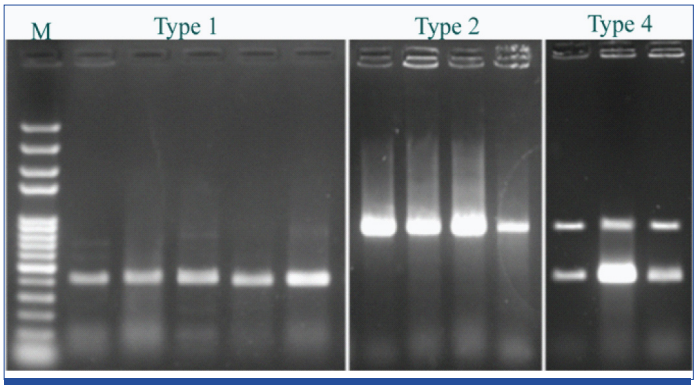



**[Table/Fig-5]:** PCR amplification of the *PVL* genes. Lane 1: Negative Control, Lane 2: 100 bp-3kb ladder, lane 3: Positive control for *PVL* genes (502bp), Lane 4-7: Positive results for *PVL* genes.

## DISCUSSION

*S. epidermidis*, especially MRSE, is one of the major causes of serious infections such as catheter-related blood stream infections [24]. Its various virulence factors and high rate of methicillin resistance among *S. epidermidis,* cause various problems for patients and healthcare providers during treatment [1]. To investigate outbreaks in nosocomial infections, determination of the source is very important that molecular typing is a valuable tool for this purpose [25].

The purpose of this study was to investigate *SCCmec* types, *PVL* gene and determining antimicrobial resistance patterns of clinical




**[Table/Fig-6]:** Positive results for SCCmec types I (415bp), II (937bp), IV (937/415 bp).

MRSE isolates collected from patient in Isfahan. Antibiotic resistance of MRSE to ciprofloxacin, cefoxitin, clindamycin, tetracycline, gentamycin, cotrimoxazole, rifampin and vancomycin using disk diffusion method was investigated. Our results showed that the prevalence of methicillin resistance among the *S. epidermidis* isolates was found to be 70 (53.8%) which is in contrast with Namvar AE et al., with 83.4% [23]. This resistance rate was lower than those found in the rest of Iran [23,26]. In a similar study carried out by Du X et al., prevalence of MRSE isolates was 86.7% among clinical isolates which are in contrast with the current study [27]. However, the prevalence of MRSE varied widely among different population and regions representing different health policies and other factors involved.

*S. epidermidis* isolates were resistant to many common antibiotics in the clinic. In antimicrobial susceptibility pattern of isolates, ciprofloxacin (81.4%) and co-trimoxazole (64%) had the highest resistance, while resistance to rifampin and gentamycin was 45.7% and 37.1% respectively. Also, all the isolates were completely sensitive to vancomycin. Overall, the rate of antibiotic resistance in our results was lower than the previous studies which can be associated with the pattern of antibiotic use in medical wards [15,26]. In the present study, *SCCmec* typing was used for detection and epidemiological investigations of isolates. Our findings show that a total of 70 MRSE isolates, *SCCmec* type were found in 88.5% (62/70) isolates. In other words, among MRSE isolates, the most common *SCCmec* type was type I, with a frequency rate of 43% (30/70), the second most frequent *SCCmec* was type IV with a rate of 34.2% (24/70) followed by type II in 11.4% (8/70) of isolates, whereas 11.4% (8/70) of the MRSE isolates could not be typed. Consistent with our results, Mertens A et al., revealed that 39.5% of *S. epidermidis* isolates carry *SCCmec* type I [28]. Also, in a study in Brazil, Machado ABMP et al., in 2013 indicated that 27.9% of *S. epidermidis* isolates harboured *SCCmec* type I, these data was similar to our results. In addition, Machado ABMP et al., showed no *SCCmec* type II in their population which is different with our finding [13]. In a study by Namvar AE et al., characterization of *SCCmec* in *S. epidermidis* identified 2 (2.1%) isolates harboured *SCCmec* type I, and the rate of *SCCmec* type IV were 42 (43.8%) which is in contrast to the results found in our study [23]. Possible reasons for the wide range of *SCCmec* type might be due to differences in geographical regions, the study population, and detection methods [29,30]. Our result is similar to studies which have shown that non-typeable *SCCmec* among *S. epidermidis* isolate's rate is higher than others [2,23]. This higher rate might be due to the presence of other *SCCmec* types [31,32]. Most of the *SCCmec* type I and IV isolates were resistant to all antibiotics excluding vancomycin, while in comparison to other types, *SCCmec* type II isolates were found to be more sensitive to gentamycin, tetracycline and rifampin. Generally, among all of *SCCmec* types, the highest antibiotic sensitivity of the isolates was against gentamycin, followed by rifampin. Our results showed, 17% (12/70) carried the *PVL* gene and these data indicated a high rate of PVL gene among *S epidermidis*. In comparison with the present study, Sani NAM et al., revealed 8% of the *S. epidermidis* isolate

had the complete operon of staphylococcal toxin genes such as *PVL* [33]. Also, in a study in Korea, PCR results for *PVL* revealed 15 (71.4%) of the *S. epidermidis* isolates carried the *PVL* genes [34]. Although, several reports demonstrate low prevalence of *PVL* gene similar to our findings which is usually associated with cutaneous infections. While, *PVL* gene for CoNS was absent in a number of studies [35,36]. Increased prevalence of *PVL* gene and *SCCmec* types among *S. epidermidis* isolates is an alarming situation. Further investigations are needed to be done on the same to prevent any complications regarding the treatment in near future.

## LIMITATION

There were several limitation to our study; first, detection of other types of *SCCmec* and their subtypes was not performed. Second, the patients participated in the study were from one hospital causing difficulty to generalize our results to other hospitals. Last, our sample size was not enough to interpret the characterization of *SCCmec* and distribution of MRSE in our region. MIC of vancomycin and other antimicrobial agents was not determined.

## CONCLUSION

Screening and isolation of MRSE-positive patients is essential in order to control the transmission of MRSE in both community and hospitals. *SCCmec* typing is useful in differentiating both and thus can be used for early diagnosis of infections.

## ACKNOWLEDGMENTS

We thank all personals at the infectious diseases research center especially Miss Shafiei R for her technical support. All the reference strains were kindly provided by the Department of Bacteriology and Virology, School of Medicine, Shiraz University of Medical Sciences Shiraz, Iran.

## REFERENCES

[1] Otto M. *Staphylococcus epidermidis*—the'accidental' pathogen. Nature Rev Microbiol. 2009;7(8):555-67.
[2] Svensson K, Hellmark B, Söderquist B. Characterization of *SCCmec* elements in methicillin-resistant *Staphylococcus epidermidis* isolated from blood cultures from neonates during three decades. Apmis. 2011;119(12):885-93.
[3] Cantey JB, Milstone AM. Bloodstream infections: epidemiology and resistance. Clin Perinatol. 2015;42(1):1-16.
[4] Piette A, Verschraegen G. Role of coagulase-negative staphylococci in human disease. Vet Microbiol. 2009;134:45-54.
[5] Diekema DJ, Pfaller MA, Schmitz FJ, Smayevsky J, Bell J, Jones RN, et al. Survey of infections due to Staphylococcus species: Frequency of occurrence and antimicrobial susceptibility of isolates collected in the United States, Canada, Latin America, Europe, and the Western Pacific region for the SENTRY Antimicrobial Surveillance Program, 1997–1999. Clin Infect Dis. 2001;32:S114–S132.
[6] Wang LF, Li JL, Ma WH, Li JY. Drug resistance analysis of bacterial strains isolated from burn patients. Genet Mol Res. 2014;13:9727–34.
[7] Wang PJ, Xie CB, Sun FH, Guo LJ, Dai M, Cheng X, et al. Molecular characteristics of methicillin-resistant *Staphylococcus epidermidis* on the abdominal skin of females before laparotomy. Int J Mol Sci. 2016;17(6):992.
[8] Hiramatsu K, Cui L, Kuroda M, Ito T. The emergence and evolution of methicillin-resistant *Staphylococcus aureus*. Trends Microbiol. 2001;9(10):486-93.
[9] Vitali LA, Petrelli D, Lamikanra A, Prenna M, Akinkunmi EO. Diversity of antibiotic resistance genes and staphylococcal cassette chromosome mec elements in faecal isolates of coagulase-negative staphylococci from Nigeria. BMC Microbiol. 2014;14:106.
[10] Havaei SA, Assadbeigi B, Esfahani BN, Hoseini NS, Rezaei N, Havaei SR. Detection of mecA and enterotoxin genes in *Staphylococcus aureus* isolates associated with bovine mastitis and characterization of Staphylococcal cassette chromosome mec (SCCmec) in MRSA strains. Iran J Microbiol. 2015;7:161.
[11] Ebrahim-Saraie HS, Motamedifar M, Sarvari J, Alfatemi SMH. Emergence of *SCCmec* Type I obtained from clinical samples in Shiraz teaching hospitals, South-West of Iran. Jundishapur J Microbiol. 2015;8:6.
[12] Turlej A, Hryniewicz W, Empel J. *Staphylococcal cassette chromosome mec* (*SCCmec*) classification and typing methods: an overview. Pol J Microbiol. 2011;60:95-103.
[13] Machado ABMP, Reiter KC, Paiva RM, Barth AL. Distribution of *staphylococcal cassette chromosome mec* (*SCCmec*) types I, II, III and IV in coagulase-negative staphylococci from patients attending a tertiary hospital in southern Brazil. J Med Microbiol. 2007;56:1328-33.
[14] Sawanobori E, Hung WC, Takano T, Hachuda K, Horiuchi T, Higuchi W, et al. Emergence of Panton-Valentine leukocidin-positive ST59 methicillin-susceptible *Staphylococcus aureus* with high cytolytic peptide expression in association with community-acquired pediatric osteomyelitis complicated by pulmonary embolism. J Microbiol Immunol. 2015;48:565-73.
[15] Ruppé E, Barbier F, Mesli Y, Maiga A, Cojocaru R, Benkhalfat M, et al. Diversity of staphylococcal cassette chromosome mec structures in methicillin-resistant *Staphylococcus epidermidis* and *Staphylococcus haemolyticus* strains among outpatients from four countries. Antimicrob Agents Chemother. 2009;53:442-49.
[16] Miragaia M, Carrico J, Thomas J, Couto I, Enright M, De Lencastre H. Comparison of molecular typing methods for characterization of *Staphylococcus epidermidis*: proposal for clone definition. J Clin Microbiol. 2008;46:118-29.
[17] Kim SJ, Park C. Panton-Valentine leukocidin and staphylococcal cassette chromosome (SSCmec) from CA-MRSA (Community-Acquired Methicillin Resistanct *Staphylococcus aureus*. Biomed Res. 2014;25:441-44.
[18] Havaei S, Moghadam SO, Pourmand MR, Faghri J. Prevalence of genes encoding bi-component leukocidins among clinical isolates of methicillin resistant *Staphylococcus aureus*. Iran J Public Health. 2010;39:8.
[19] Alfatemi SMH, Motamedifar M, Hadi N, Saraie HSE. Analysis of virulence genes among methicillin resistant *Staphylococcus aureus* (MRSA) strains. Jundishapur J Microbiol. 2014;7:6.
[20] CLSI. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Third Informational Supplement. CLSI document M100-S23. Wayne, PA: Clinical and Laboratory Standards Institute; 2013.
[21] Oliveira CF, Paim TG, Reiter KC, Rieger A, D'azevedo PA. Evaluation of four different DNA extraction methods in coagulase-negative Staphylococci clinical isolates. Rev Inst Med Trop Sao Paulo. 2014;56:29-33.
[22] Azimian A, Havaei SA, Fazeli H, Naderi M, Ghazvini K, Samiee SM, et al. Genetic characterization of a vancomycin-resistant *Staphylococcus aureus* isolate from the respiratory tract of a patient in a university hospital in northeastern Iran. J Clin Microbiol. 2012;50:3581-85.
[23] Namvar AE, Havaei SA, Moghim S, Lari AR. Characterization of *Staphylococcus epidermidis* isolates from hospitalized patients in Isfahan and Tehran teaching hospitals. Mol Genet Microbiol Virol. 2014;29(4):216-19.
[24] Cherifi S, Byl B, Deplano A, Nagant C, Nonhoff C, Denis O, et al. Genetic characteristics and antimicrobial resistance of *Staphylococcus epidermidis* isolates from patients with catheter-related bloodstream infections and from colonized healthcare workers in a Belgian hospital. Ann Clin Microbiol Antimicrob. 2014;13:1.
[25] Havaei SA, Ghanbari F, Rastegari AA, Azimian A, Khademi F, Hosseini N, et al. Molecular typing of hospital-acquired *Staphylococcus aureus* isolated from Isfahan, Iran. Int Sch Res Notices. 2014;2014:185272.
[26] Pishva E, Havaei SA, Arsalani F, Narimani T, Azimian A, Akbari M. Detection of methicillin-resistance gene in *Staphylococcus epidermidis* strains isolated from patients in Al-Zahra Hospital using polymerase chain reaction and minimum inhibitory concentration methods. Adv Biomed Res. 2013;2:23.
[27] Du X, Zhu Y, Song Y, Li T, Luo T, Sun G, et al. Molecular analysis of *Staphylococcus epidermidis* strains isolated from community and hospital environments in China. PLoS One. 2013;8(5):e62742.
[28] Mertens A, Ghebremedhin B. Genetic determinants and biofilm formation of clinical *Staphylococcus epidermidis* isolates from blood cultures and indwelling devises. Eur J Microbiol Immunol(Bp). 2013;3(2):111-19.
[29] Ito T, Kuwahara-Arai K, Katayama Y, Uehara Y, Han X, Kondo Y, et al. staphylococcal cassette chromosome mec (*SCCmec*) analysis of MRSA. Methods Mol Biol. 2014;1085:131-48.
[30] Kang CK, Cho JE, Choi YJ, Jung Y, Kim NH, Kim CJ, et al. Agr dysfunction affects staphylococcal cassette chromosome mec type-dependent clinical outcomes in methicillin-resistant *Staphylococcus aureus* bacteremia. Antimicrob Agents Chemother. 2015;59(6):3125-32.
[31] Soroush S, Jabalameli F, Taherikalani M, Amirmozafari N, Fooladi AA, Asadollahi K, et al. Investigation of biofilm formation ability, antimicrobial resistance and the staphylococcal cassette chromosome mec patterns of methicillin resistant Staphylococcus epidermidis with different sequence types isolated from children. Microb Pathog. 2016;93:126-30.
[32] Abadi MIM, Moniri R, Khorshidi A, Piroozmand A, Mousavi SG, Dastehgoli K, et al. Molecular characteristics of nasal carriage methicillin-resistant coagulase negative Staphylococci in School students. Jundishapur J Microbiol. 2015;8(6):e18591.
[33] Sani NAM, Sapri HF, Neoh H-m, Hussin S. First report on the molecular epidemiology of Malaysian Staphylococcus epidermidis isolated from a University Teaching Hospital. BMC Res Notes. 2014;7:597.
[34] Park HK, Woo SY, Jung YJ, Lee EO, Cha JE, Park HS, et al. Detection of virulence genes of *Staphyloccus aureus* and *Staphylococcus epidermidis* isolated from suprapubic urine from infants with fever. J Bacteriol Virol. 2008;38(4):189-96.
[35] Abimanyu N, Krishnan A, Murugesan S, Subramanian GK, Gurumurthy S, Krishnan P, et al. Use of triplex pcr for rapid detection of pvl and differentiation of mrsa from methicillin resistant coagulase negative staphylococci. J Clin Diagn Res. 2013;7(2):215-18.

[36] McClure JA, Conly JM, Lau V, Elsayed S, Louie T, Hutchins W, et al. Novel multiplex PCR assay for detection of the staphylococcal virulence marker Panton-Valentine leukocidin genes and simultaneous discrimination of methicillin-susceptible from-resistant staphylococci. J Clin Microbiol. 2006;44(3):1141-43.

**PARTICULARS OF CONTRIBUTORS:**
1. Department of Microbiology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran.
2. Department of Microbiology, Ahar Branch, Islamic Azad University, Ahar, Iran.
3. Nosocomial Infection Research Center, Isfahan University of Medical Sciences, Isfahan, Iran.
4. Department of Microbiology, Ahar Branch, Islamic Azad University, Ahar, Iran.
5. Nosocomial Infection Research Center, Isfahan University of Medical Sciences, Isfahan, Iran.
6. Acquired Immunodeficiency Research Center, Isfahan University of Medical Sciences, Isfahan, Iran.
7. Infectious Diseases and Tropical Medicine Research Center, Isfahan University of Medical Sciences, Isfahan, Iran.
8. Nosocomial Infection Research Center, Isfahan University of Medical Sciences, Isfahan, Iran.

**NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:**
Dr. Seyed Asghar Havaei,
Nosocomial Infection Research Center, Isfahan University of Medical Sciences, Isfahan, Iran.
E-mail: havaei@med.mui.ac.ir

**FINANCIAL OR OTHER COMPETING INTERESTS:** None.

Date of Submission: **Nov 20, 2016**
Date of Peer Review: **Jan 12, 2017**
Date of Acceptance: **Apr 20, 2017**
Date of Publishing: **Jul 01, 2017**