Evaluation of various staphylococcal cassette chromosome mec (SCCmec) types in *Staphylococcus epidermidis* invasive strains from hospitalised patients in Iran

Valutazione di tipi diversi di cassetta cromosomica stafilococcica mec (SCCmec) in ceppi invasivi di Staphylococcus epidermidis *isolati da pazienti ospedalizzati in Iran*

Seyed Asghar Havaei[1], Amirmorteza Ebrahimzadeh Namvar[1], Sharareh Moghim[1], Abdolaziz Rastegar Lari[2]

[1]Department of Microbiology, School of Medicine, Isfahan University of Medical Sciences Isfahan, Iran;
[2]Department of Microbiology Faculty of Medicine, Iran University of Medical Sciences, Tehran, Iran

INTRODUCTION

Staphylococcus epidermidis has recently become established as a major cause of nosocomial infections particularly in catheter-associated bacteraemia, prosthetic valve endocarditis (PVE) and immunocompromised patients in different health care units such as NICU and ICU [1, 2]. Due to the emergence of multidrug-resistant strains, especially to β-lactam antibiotics, the mortality and morbidity due to this micro-organism has increased. A kind of low affinity penicillin-binding protein (PBP2α), which is encoded by the mecA gene, located in the staphylococcal cassette chromosome mec (SCCmec), mediates the resistance to methicillin. Resistance to the above antibiotic was initially detected in *Staphylococcus aureus* in 1961. Thereafter other methicillin-resistant strains which were associated with global outbreaks of nosocomial infections became increasingly common [3, 4]. SCCmec is a mobile genetic element, which consists of three main regions, the mec complex (mecA, IS431, mecI and mecR), the ccr gene complex (ccrA, ccrB and ccrC) and the Joining region (J1-J3) [5, 6]. The SCCmec elements were first identified in Japan (1999), and then various types were reported worldwide [7, 8]. To date, five main types (I-V) of SCCmec have been detected in coagulase-negative staphylococci (CNS) [9]. Despite the high transmission ability of the gene between different species of staphylococci the origin of SCCmec is still unknown. According to the studies conducted, the mecA-positive strains of *S. epidermidis* are regarded as a capable source of SCCmec elements [10]. Due to widespread studies on *S. aureus* SCCmec elements structure and the lack of research in the field of *S. epidermidis*, the aim of this study was to investigate the prevalence of SCCmec types and evaluate the antibiotic profile assay in invasive strains isolated from clinical samples.

Corresponding author
Amirmorteza Ebrahimzadeh Namvar
E-mail: amirmorteza.namvar@gmail.com

MATERIALS AND METHODS

Bacterial isolates

In the current study 145 strains of *S. epidermidis* isolates were collected from different clinical samples in which 70 isolates were determined as invasive strains under aseptic technique after being isolated three times and cultured with an acceptable colony-forming unit. The isolates were gathered from blood cultures, catheters, wounds and CSF of

patients hospitalised at the Alzahra and H. Rasool university hospitals in Isfahan and Tehran in 2014.

Phenotypic identification
Standard microbiological tests, namely blood agar culture, Gram staining, catalase, coagulase, and susceptibility to polymyxin B (MAST, Merseyside, England), were used for isolate confirmation. Finally, the micro-tubes containing 15% glycerol were subsequently stored at -20°C.

Antimicrobial profile assay
In antimicrobial resistance assay by using the disc diffusion method the following discs were used: ciprofloxacin (5 µg), clindamycin (2 µg), gentamicin (10 µg), cefoxitin (30 µg), tetracycline (30 µg), oxacillin (1 µg), vancomycin (30 µg), sulfamethoxazole (23.75 µg) + trimethoprim (1.25 µg), levofloxacin (5 µg) and rifampicin (5 µg) (MAST, Merseyside, England) based on the Clinical and Laboratory Standards Institute (CLSI) (2010). Also *Staphylococcus aureus* ATCC 25923 was preferred as a quality control strain.

DNA extraction
Purification of genomic DNA was prepared using a QIAamp DNA minikit according to the manufacturers´ recommendations.

Detection of the mecA gene
The specific primers for amplification of the 547 bp fraction of the mecA gene by PCR molecular test (F: 5´-TGGTATGTGGAAGTTAGATTGG-3´ & R: 5´-AACGATTGTGACACGATAGC-3´) were designed with primer designer software from GenBank sequences (HE978798). Forty cycles of PCR were performed in a final volume of 25µl using EmeraldAmp® MAX PCR Master Mix (Takara, Japan) with the following protocol: initial denaturation at 94°C for 5 min, denaturation, at 94°C for 45 seconds, annealing at 53°C for 45 seconds, extension for 45 seconds at 72°C and a final extension step at 72°C for 5 min.

Determination of SCCmec types
The oligonucleotide primers which were described previously by Boye et al. were used for verifying the various types of SCCmec elements [11].

RESULTS

A total of 70 *S. epidermidis* invasive strains isolated from hospitalised patients were studied after being isolated three times and cultured in conventional microbiological medium with the acceptable colony count. The specimens included blood cultures (37.5%), eye wounds (20%), intravenous catheter (12.5%) and ear wounds (10%). Among the isolates, 60% belonged to males and 40% to females. The results obtained from antibiotic susceptibility analysis (Figure 1) were as follows: gentamicin (57.5%), cefoxitin (87.1%), ciprofloxa-

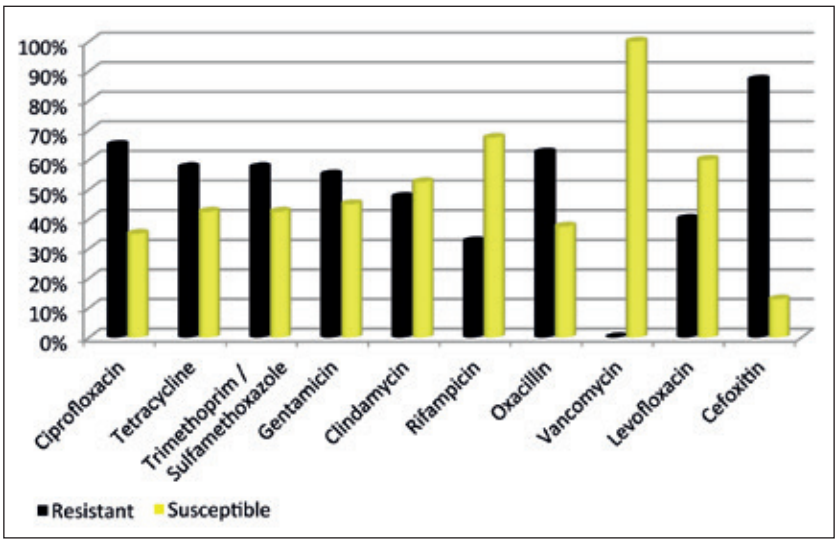

Figure 1 - Antibiotic susceptibility patterns of *S. epidermidis* isolates from hospitalized patients.

cin (65%), oxacillin (62.5%), vancomycin (0%), tetracycline (55%), clindamycin (47.5%), trimethoprim / sulfamethoxazole (57.5%), rifampicin (32.5%) and levofloxacin (40%). The presence of the mecA gene was subsequently determined by PCR molecular test in which 87.1% of all isolates were found to be positive (Figure 2). In this research five (I-V) different types of SCCmec were investigated with the following details: SCCmec I was detected in 3 isolates, SCCmec II in 5 isolates, SCCmec III in 22 isolates, SCCmec IV in 27 isolates and SCCmec V in 4 isolates (Figure 3).

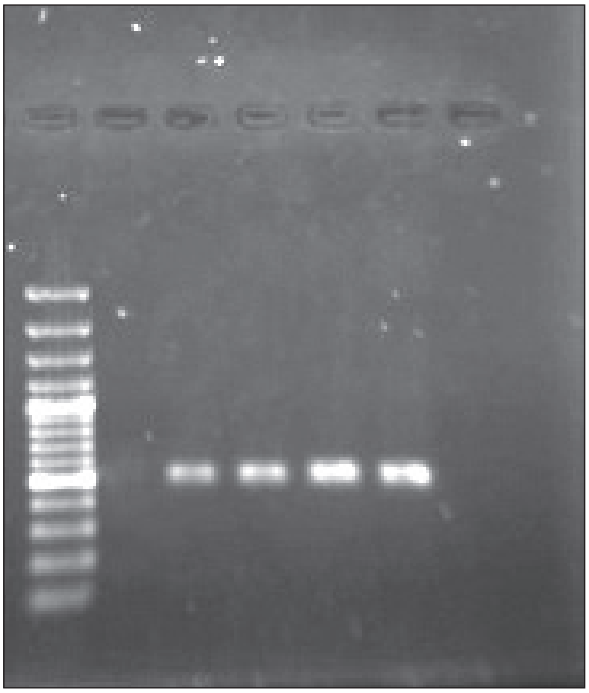

Figure 2 - (1%) gel agarose electrophoresis showing the results of the PCR molecular test for amplification of mecA gene in *S. epidermidis* isolates. Lane 1: M-molecular size marker 100 bp (Fermentas); lane 2: negative control; lane 3: positive control; lane 4, 5 and 6: positive isolates (547 bp).

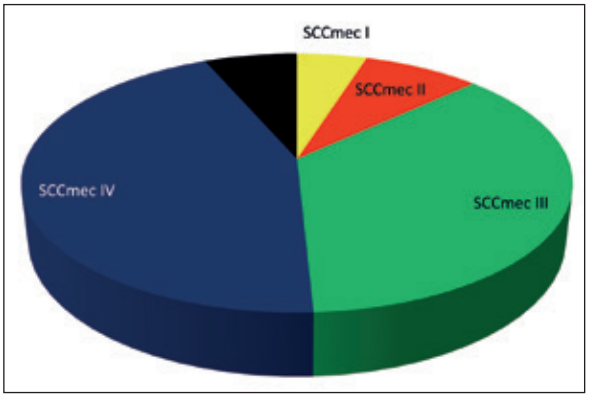

Figure 3 - The prevalence of various SCCmec types.

DISCUSSION

Coagulase-negative staphylococcus species, especially *S. epidermidis*, are known as the normal flora of human skin, the nose and the other mucosal surfaces. Hence these micro-organisms have, in the past, rarely been considered a major cause of human illnesses. At the same time, the widespread use of medical equipment such as catheters, medical implants and related devices have led to *S. epidermidis* being currently one of the most significant causes of nosocomial infections. Fifty-nine percent of postoperative infections and also 63% of blood culture isolated bacteria from neonatal units belong to this bacteria [12].

It is worth pointing out that neonates, the immunocompromised, and hospitalised patients are considered the main infected groups. Presence of various virulence factors and resistance to β-lactam drugs such as penicillin and methicillin has increased the infections and stability of this micro-organism in different parts of the human body [13]. Antibiotic resistance is provided due to the transferring ability of genetic elements, existence of virulence factors and indiscriminate usage of antibiotics. In a study conducted in 1999 by De Giusti resistance to methicillin was found to be 48.6%. In numerous studies, primers which were used for determining the mecA gene were designed based on *S. aureus* strains [14].

However, in our research *S. epidermidis* was applied as the basis of specific designed primers. The frequency of the mecA gene in our isolates was 87.1% while in studies by Wang et al. and Li et al., this frequency was reported as 70% and 96.25%, respectively [15, 16]. In the antibiotic susceptibility analysis, resistance to oxacilin and cefoxitin was found to be 62.5% and 87.1%, respectively. In addition, the cefoxitin disc result was similar to PCR molecular test. According to CLSI standard guidelines, Machado et al. and our results, the value of the cefoxitin disc to diagnose the *S. epidermidis* resistant strains is significant [17]. Dif-

ferent structures of various SCCmec elements are one of the most functional tools for epidemiological typing methods in studies on methicillin-resistant *Staphylococcus epidermidis* (MRSE) strains and in evaluating the relevant clones. Investigators classify types IV and V as community-acquired MRSE (CA-MRSE) [9, 18]. In our study five different types of SCCmec were recognized: 4.9% were found to harbour type I, 8.2% type II, 36.1% type III, 44.3% type IV, and 6.5% type V, although Miragaia and colleagues in 2008 determined type IV (41%), type III (27%), type V (6%) and both types I & II (4%) [19]. In Bouchami's study in 2011 the results indicated that type IV (31%) and type III of SCCmec (24.5%) had the highest percentage [20]. Despite our results, in which type IV of SCCmec had a significant percentage, this circumstance is not always easily comprehensible because in many studies the other SCCmec types may show the highest percentages. Based on SCCmec different types, our isolates were classified in CA-MRSE. Thus, according to the virulence ability of CA-MRSE strains, the prevalence of MRSE-related infections in hospitalised patients is not unexpected. Since CNS are reported as a major cause of hospital infections, molecular typing methods like SCCmec typing would be helpful to control and prevent bacterial infections.

Acknowledgements
This study was funded in part by a grant from the Isfahan University of Medical Sciences (Grant no. 392003).

Keyword: Staphylococcus epidermidis, mecA gene, SCCmec typing, nosocomial infections.

SUMMARY

Staphylococcus epidermidis is known to be a major cause of nosocomial infections particularly in catheter-associated bacteraemia, prosthetic valve endocarditis (PVE) and immunocompromised patients in different health care units. The emergence of multidrug-resistant strains, especially to β-lactam antibiotics such as methicillin, has increased the mortality due to *S. epidermidis*. A kind of low affinity penicillin-binding protein (PBP2α), which is encoded by the mecA gene that is located in the staphylococcal cassette chromosome mec (SCCmec), mediates the resistance to methicillin. The aim of this study was to investigate the prevalence of SCCmec types and evaluate the antibiotic profile assay in invasive strains isolated from clinical samples. The study focused on invasive strains, determining the antimicrobial resistance profile, designing new primers for detection of the mecA gene and SCCmec typing with the multiplex PCR method. By using the PCR molecular test, 87.1% of all isolates were found to be positive for the mecA gene. In SCCmec typing, different types (I-V) were identified, in which SCCmec type I was detected in 3 isolates, SCCmec type II in 5 isolates, SCCmec type III in 22 isolates, SCCmec type IV in 27 isolates and SCCmec type V was distinguished in 4 isolates. Since coagulase-negative staphylococci are reported as a major cause of hospital infections, molecular typing methods like SCCmec typing would be a helpful method to control and prevent bacterial infections.

RIASSUNTO

Staphylococcus epidermidis *è una delle cause principali di infezioni nososcomiali, soprattutto batteriemie cateterecorrelate ed endocarditi su valvola protesica (PVE) e in pazienti immunocompromessi in diversi reparti. L'emergere di ceppi multi resistenti, soprattutto ad antibiotici beta-lattamici quale la meticillina, ha determinato un aumento della mortalità attribuibile a S. epidermidis. La resistenza alla meticillina è mediata da un tipo di proteina di legame alla penicillina a bassa affinità (PBP2α) che è codificata dal gene mecA, localizzato nella cassetta cromosomica stafilococcica mec (SCCmec). Questo studio è stato condotto al fine di analizzare la prevalenza dei tipi di SCCmec e valutare il profilo di sensibilità agli antibiotici in ceppi invasivi isolati da campioni clinici. Lo studio ha preso in esame ceppi invasivi, determinandone il profilo di resistenza antibiotica e mettendo a punto nuovi primers per la rilevazione del gene mecA; la tipizzazione di SCCmec è stata effettuata con un metodo PCR multiplex. Utilizzando il test molecolare PCR, l'87,1% di tutti gli isolati è risultato positivo per la presenza del gene mecA. La tipizzazione di SCCmec ha consentito di identificare tipi differenti (I-V), di cui SCCmec tipo I è stato rilevato in 3 isolati, SCCmec tipo II in 5 isolati, SCCmec tipo III in 22 isolati, SCCmec tipo IV in 27 isolati e SCCmec tipo V in 4 isolati. Considerato che gli stafilococchi coagulasi negativi costituiscono una delle principali cause di infezioni nosocomiali, i metodi di tipizzazione molecolare, quale ad esempio la tipizzazione di SCCmec, potrebbero costituire un metodo utile per il controllo e la prevenzione delle infezioni batteriche.*

References

[1] Curtis C., Shetty N. Recent trends and prevention of infection in the neonatal intensive care unit. *Curr. Opin. Infect. Dis.* 21, 350-356, 2008.
[2] Falcone M., Campanile F., Giannella M., Borbone S., Stefani S., Venditti M. *Staphylococcus haemolyticus* endocarditis: clinical and microbiologic analysis of 4 cases. *Diagn. Microbiol. Infect. Dis.* 57, 325-331, 2007.
[3] Chambers H.F., Deleo F.R. Waves of resistance: *Staphylococcus aureus* in the antibiotic era. *Nat. Rev. Microbiol.* 7, 629-641,2009.
[4] Deurenberg R.H., Stobberingh E.E. The evolution of *Staphylococcus aureus. Infect. Genet. Evol.* 8,747-763, 2008.
[5] Chongtrakool P., Ito T., Ma X.X., et al. Staphylococcal cassette chromosome mec (SCCmec) typing of methicillin-resistant *Staphylococcus aureus* strains isolated in 11 Asian countries: a proposal for a new nomenclature for SCCmec elements. *Antimicrob. Agents. Chemother.* 50, 1001-1012, 2006.
[6] Oliveira D.C., Milheiric O.C., de Lencastre H. Redefining a structural variant of staphylococcal cassette chromosome mec, SCCmec type VI. *Antimicrob. Agents. Chemother.* 50, 3457-3459, 2006.
[7] Kondo Y., Ito T., Ma X.X., et al. Combination of multiplex PCRs for staphylococcal cassette chromosome mec type assignment: rapid identification system for mec, ccr, and major differences in junkyard regions. *Antimicrob. Agents. Chemother.* 51, 264-274, 2007.
[8] Ito T., Katayama Y., Hiramatsu K. Cloning and nucleotide sequence determination of the entire mec DNA of pre-methicillin-resistant *Staphylococcus aureus* N315. *Antimicrob. Agents Chemother.* 43, 1449-1458,1999.
[9] Wisplinghoff H., Rosato A.E., Enright M.C., Noto M., Craig W., Archer G.L. Related clones containing SCCmec type IV predominate among clinically significant *Staphylococcus epidermidis* isolates. *Antimicrob. Agents Chemother.* 47, 3574-3579, 2003.
[10] Mongkolrattanothai K., Boyle S., Murphy T.V., Daum R.S. Novel non-mecA-containing staphylococcal chromosomal cassette Composite Island containing pbp4 and tagF genes in a commensal staphylococcal species: a possible reservoir for antibiotic resistance islands in *Staphylococcus aureus*. *Antimicrob. Agents Chemother.* 48, 1823-1836, 2004.
[11] Boye K., Bartels M.D., Andersen I.S., Møller J.A., Westh H. A new multiplex PCR for easy screening of methicillin-resistant *Staphylococcus aureus* SCCmec types I–V. *Clin. Microbiol. Infect.* 13, 725-727, 2007.
[12] Smith I.M. *Staphylococcus epidermidis* septicemia: 1974-1978 compared to 1949-1958, In Staphylococci and staphylococcal infections. (J. Jelijasze- wicz, Ed) 1981, 765-772. Gustav Fischer Verlag, Stuttgart, Federal Republic of Germany.
[13] Berger-Bachi B., Rohrer S. Factors influencing methicillin resistance in staphylococci. *Arch. Microbiol.* 178, 165-171, 2002.
[14] De Giusti M. Phenotypic detection of nosocomial mecA positive coagulase negative staphylococci from neonates. *J. Antimicrob. Chemother.* 44, 351-358, 1999.
[15] Wang X.M., Noble L., Kreiswirth B.N., et al. Evaluation of a multilocus sequence typing system for *Staphylococcus epidermidis*. *J. Med. Microbiol.* 52, 989-998, 2003.
[16] Li M., Wang X., Gao Q., Lu Y. Molecular characterization of *Staphylococcus epidermidis* strains isolated from a teaching hospital in Shanghai, China. *J. Med. Microbiol.* 58, 456-461, 2009.
[17] Machado A.B., Reiter K.C., Paiva R.M., Barth A.L. 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.* 56, 1328-1333, 2007.
[18] Hisata K., Kuwahara-Arai K., Yamanoto M., et al. Dissemination of methicillin-resistant staphylococci among healthy Japanese children. *J. Clin. Microbiol.* 43, 3364-3372, 2005.
[19] Miragaia M., Carric¸o J.A., Thomas J.C., Couto I., Enright M.C., de Lencastre H. Comparison of molecular typing methods for characterization of *Staphylococcus epidermidis*: proposal for clone definition. *J. Clin. Microbiol.* 46,118-129, 2008.
[20] Bouchami O., Achour W., Mekni M.A., Rolo J., Ben Hassen A. Antibiotic resistance and molecular characterization of clinical isolates of methicillin-resistant coagulase-negative staphylococci isolated from bacteremic patients in oncohematology. *Folia Microbiol.* 56, 122-130, 2011.