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

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INTRODUCTION

Staphylococcus epidermidis has recently become established as a major cause of nosocomial infections particularly in catheter-associated bacteremia, 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, mecl 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.

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), 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 manufacturer’s 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.

**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%), ciprofloxacin (57.5%), clindamycin (20%), and oxacillin (20%).

![Figure 1 - Antibiotic susceptibility patterns of S. epidermidis isolates from hospitalized patients.](image-url)
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).

**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 oxacillin 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].
Evaluation of various staphylococcal cassette chromosome mec (SCCmec) types

Staphylococcus epidermidis is known to be a major cause of nosocomial infections, particularly in catheter-associated bacteremia, prosthetic valve endocarditis (PVE) and immunocompromised patients in different health care units. The emergence of multidrug-resistant strains, especially to \(\beta\)-lactam antibiotics such as methicillin, has increased the mortality due to \textit{S. epidermidis}. A kind of low affinity penicillin-binding protein (PBP2\(\alpha\)), 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 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.

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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 bacteremia, prosthetic valve endocarditis (PVE) and immunocompromised patients in different health care units. The emergence of multidrug-resistant strains, especially to \(\beta\)-lactam antibiotics such as methicillin, has increased the mortality due to \textit{S. epidermidis}. A kind of low affinity penicillin-binding protein (PBP2\(\alpha\)), 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 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 nosocomiali, soprattutto batteriemie catetere-correlate 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 \textit{S. epidermidis}. La resistenza alla meticillina è mediata da un tipo di proteina di legame alla penicillina a bassa affinità (PBP2\(\alpha\)) che è codificata dal gene mecA, localizzato nella cassetta cromosomica stafillococcica 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 stafilococi coagulasi negativi sono riferiti come 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