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SHORT COMMUNICATION

Characterization of Staphylococcal Cassette Chromosome *mec* (SCC*mec*) in Methicillin-Resistant *Staphylococcus epidermidis* Strains Isolated from Biomaterial-Associated Infections and their Antibiotic Resistance Patterns

EWA SZCZUKA[1]*, KAROLINA BOSACKA[2] and ADAM KAZNOWSKI[1]

[1] Department of Microbiology, Institute of Experimental Biology, Faculty of Biology, Adam Mickiewicz University, Poznań, Poland
[2] Department of Microbiological and Laboratory Diagnostics, Bacteriological Laboratory, Regional Hospital in Poznań, Poznań, Poland

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Abstract

This work aims to provide an insight into staphylococcal cassette chromosome *mec* elements and antibiotic resistance in clinical isolates of *Staphylococcus epidermidis*. The dominating type was SCC*mec* – IV. Fifteen isolates were assigned to SCC*mec* type III, two isolates to SCC*mec* type II. Most isolates were resistant to at least three of the non-β-lactam antibiotics tested. None of the strains exhibited resistance to new generation antibiotics, such as daptomycin and linezolid. Also, none of these strains showed resistance to tigecycline and only four strains were resistant to rifampin *i.e.* antibiotics which are very efficient in treating biofilm-associated infections.

Key words: *Staphylococcus epidermidis*, SCC*mec* type, antibiotic resistance

Among coagulase-negative staphylococci (CoNS), *Staphylococcus epidermidis* is the leading cause of hospital-acquired and biomaterial-associated infections. This bacterium can be responsible for endocarditis, peritonitis, bone and joint infections, septicaemia and bacteremia (Voung and Otto, 2002). The main virulence factor associated with *S. epidermidis* is the ability to form biofilm on implanted medical devices or damaged tissues. Strains belonging to this species have been particularly efficient at developing resistance to antimicrobial agents, which is due in part to the presence of the mobile genetic elements caring resistance genes (Schoenfelder *et al*., 2010). The *mec*A gene, which encodes PBP2a, a transpeptidase with a low affinity for beta-lactam antibiotics, is carried on a mobile genetic element called the staphylococcal chromosome *mec* (SCC*mec*). In addition, resistance gene for macrolides, tetracyclines and aminoglycosides can accrue on the SCC*mec* cassette. This element is bound by terminal inverted repeat sequences (IR) and integrated at the 3' end of the *orf*X gene, which is located near the origin of replication in the chromosome. Eleven types (I to XI) of SCC*mec* have been assigned for staphylococci based on the composition of the *ccr* gene complex and the class of the *mec* gene complex (Ito *et al*., 2001; 2004; IWG, 2009; Shore *et al*., 2013; Turlej *et al*., 2011). The *mec* gene complex is composed of *mec*A gene, intact or truncated sets of regulatory genes (*mec*RI and *mec*I), hypervariable region (HVR) and associated insertion sequence (IWG, 2009). The *ccr* gene complex encodes the reccombinase that plays an important role in integration and excision of SCC*mec* from the chromosome. Three district *ccr* genes, *ccr*A, *ccr*B and *ccr*C have been described in staphylococci strains. In addition to the *ccr* and *mec* gene complex, SCC*mec* cassette contains various mobile genetic elements (MGE), *e.g.* insertion sequences, transposons and plasmids, which are located in the joining regions (IWG, 2009). The occurrence of a very similar SCC*mec* cassette in different species provided evidence that genetic transfer of this element occurs between species in nature (Barbier *et al*., 2010; Hanssen and Ericson Sollid, 2006; Smyth *et al*., 2010). Moreover, Bloemendaal *et al.* (2010) demonstrated *in vitro* the transfer of SCCmec IV from *S. epidermidis* to the most virulent staphylococcal species, *Staphylococcus aureus*.

The aim of this study was to investigate the distribution of SCC*mec* types among *S. epidermidis* strains

* Corresponding author: E. Szczuka, Department of Microbiology, Institute of Experimental Biology, Faculty of Biology, Adam Mickiewicz University, Poznań, Poland; **e-mail: ewasz@amu.edu.pl**

recovered from biomaterial-associated infections and analyse the antibiotic resistance patterns of these strains.

Sixty five CoNS collected from hospitalized patients were analyzed. Forty-six strains were isolated from the catheter-related bloodstream infection of hospitalized patients, which were regarded as causative agents of blood stream-infections (the same strain isolated from blood culture from the catheter and at the same time from blood culture by venous puncture). Nine strains were isolated from infection of the prosthesis, ten from peritoneal fluid. Isolates were identified by using the Vitek 2 system (bioMérieux, France).

The bacterial genomic DNA was isolated from clinical isolates using the Genomic DNA Plus kit (A&A Biotechnology, Poland). The SCC*mec* types were identified using multiplex PCR (Zhang *et al*., 2005). The amplification products were electrophoresed in 1.5% agarose gel. The gels were stained with ethidium bromide, visualized on a UV light transilluminator, and documented with V.99 Bio-Print system (Vilber Lourmat, Torcy, France).

Resistance to β-lactams were determined by the cefoxitin (30 μg) screen test. Susceptibility to the following antibiotical agents: fluoroquinolones (ciprofloxacin, levofloxacin, moxifloxacin, norfloxacin), aminoglycosides (gentamicin, tobramycin), glycopeptides (teicoplanin, vancomycin), macrolides and lincosamides (clindamycin, erythromycin), tetracyclines (tetracycline, tigecycline) and others (linezolid, rifampin, trimethoprim-sulfamethoxazole) was performed using Vitek 2 system (bioMérieux, France) according to EUCAST recommendations (http://www.eucast.org/clinical_breakpoints). Minimum inhibitory concentrations (MICs) of daptomycin were determined by microdilution method in Mueller-Hinton broth, supplemented to yield final concentration of 50 mg/l calcium (EUROCAST, 2007). Results were read after incubation at 37°C for 18–24 h. Susceptibility to daptomycin was defined as MIC value of ≤ 1 mg/l.

Tests were performed using software Excel (2010, Microsoft). The Pearson test was used to analyze correlation between SCCmec types and the resistance to different antibiotics. A P-value of < 0,05 was considered significant.

We have previously documented the occurrence of biofilm-associated genes in the majority of clinical *S. epidemidis* as well as their ability to form biofilm structures *in vitro* (Szczuka and Kaznowski, 2014). This work aims to provide an insight into staphylococcal cassette chromosome *mec* elements and antibiotic resistance. Among these strains, 82% were multiresistant. Resistance to erythromycin, clindamycin, and tetracycline was found in 45 (69%), 43 (66%) and 35 (54%) of the isolates, respectively. Twenty four (37%) were resistant to ciprofloxacin, twenty one (32%) to gentamicin and nineteen (29%) to trimethoprim-sulfamethoxazole. None of the strains exhibited resistance to glycopeptides. However, only four strains were resistant to rifampin and all were susceptible to tigecycline, antibiotics which are very effective in the treatment of biofilm-associated infections. All clinical strains were susceptible to linezolid, even though a few isolates of linezolid-resistant *S. epidermidis* were reported elsewhere (Hong *et al*., 2007; Treviño *et al*., 2009; Bonilla *et al*., 2010; Gu *et al*., 2013). In addition, all strains were susceptible to the new agent daptomycin, which demonstrated excellent *in vitro* activity against bacteria embedded in biofilms (Stewart *et al*., 2009).

It is believed that the increasing resistance of *S. epidermidis* to methicillin and other beta-lactam antibiotics is due to the presence of the SCC*mec,* which can be easily transferred between staphylococci strains, especially in biofilm structures (Garza-Gonzalez *et al*., 2010a; 2010b). Sixty two *S. epidermidis* isolates were classified into three SCC*mec* types. Forty five (69%) *S. epidermidis* isolates harbour SCC*mec* type IV, which is believed to be the most mobile version of this element. Results of this study are in agreement with previously reported data, which indicated that type SCC*mec* type IV was the most prevalent in *S. epidermidis* strains among adults treated in a French hospital (Barbier *et al*., 2010; Garza-Gonzalez *et al*., 2010a; 2010b). Also SCC*mec* type IV dominated among *S. epidermidis* isolated from outpatients living in Algeria, Mali, Moldavia and Cambodia (Ruppé *et al*., 2009). In contrast, Li *et al.* (2009) found that only two out of 38 *S. epidermidis* strains recovered from patients treated in China carried SCC*mec* type IV, whereas SCC*mec* type III was the most prevalent. Our results indicate that 15 *S. epidermidis* strains (23%) carried SCC*mec* type III. Only two isolates harboured SCC*mec* type II. None of the isolates carried type I. Our results demonstrated that the strains harbouring SCC*mec* cassette type III were in a significantly higher proportion resistant to non beta-lactam drugs, except rifampin as compared to isolates with SCC*mec* type IV (Table I). It could be explained by the presence of several resistance genes in the

Table I
Association between SCC*mec* types and resistance patterns to selected antimicrobial agents

Antimicrobial agents	Number of resistant isolates (%)		p-value
	SCC*mec* type III (n = 15)	SCC*mec* type IV (n = 45)	
ciprofloxacin	11 (73)	11 (24)	< 0.001
clindamycin	13 (86)	27 (60)	0.031
gentamicin	9 (60)	7 (15)	< 0.001
tetracycline	14 (93)	16 (35)	< 0.001
trimethoprim/ ulfamethoxazole	9 (60)	10 (22)	< 0.001

SCC*mec* cassettes type III. The distribution of SCC*mec* types among *S. epidermidis* are comparable to the results of the studies conducted by Wisplinghoff *et al.* (2003), but different from the findings reported by Svensson *et al.* (2011). It has been reported that only three isolates carried known types of SCC*mec* – type III, whereas many strains contained multiple copies of *ccr* gene complexes and one class of *mec* gene complex. It is thought that the presence of different types of *ccr* complex in SCC*mec* elements might be due to rearrangements of types of SCC*mec* in bacterial cells (Hanssen *et al*., 2006). In our studies, only three strains could not be assigned to known SCC*mec* types because *ccr* gene in these strains could not be determined. These strains contained *mec* complex B.

In conclusion, our results demonstrate the conservation of SCC*mec* element of *S. epidermidis* clinical isolates. These strains constitute a reservoir of SCC*mec* type IV. Although we found that the majority of *S. epidermidis* strains showed resistance to several antibiotics, no isolate showed resistance to daptomycin and tigecycline and only few isolates were resistant to rifampin which are the most efficient antibiotics against *S. epidermidis* biofilm-associated infections.

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