Diversity of staphylococcal cassette chromosome mec structures in coagulase-negative staphylococci and relationship to drug resistance

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The objective of this study was to determine the distribution of staphylococcal cassette chromosome mec (SCCmec) elements in meticillin-resistant coagulase-negative staphylococci (MR-CoNS) isolated from a tertiary-care hospital in Mexico and to examine the relationship to drug resistance. Fifty selected MR-CoNS isolates collected from catheters (n = 15), blood (n = 15), bone (n = 9), bronchial lavage (n = 2) and urine (n = 2) and one isolate each from an abscess, cerebrospinal fluid, eye, pleural effusion, synovial fluid, tracheal aspirate and wound secretion were examined. Susceptibility testing was performed by the broth microdilution method. SCCmec types were determined by multiplex PCR and PFGE was carried out as described previously for Staphylococcus aureus. Among the MR-CoNS strains studied, the most frequently isolated species were Staphylococcus epidermidis (n = 26) and Staphylococcus haemolyticus (n = 13). Staphylococcus cohnii (n = 5), Staphylococcus hominis (n = 3), Staphylococcus sciuri (n = 1), Staphylococcus pasteuri (n = 1) and the recently described species Staphylococcus pettenkoferi (n = 1) were also identified. The most frequent MR-CoNS genotype identified was SCCmec type IVa in S. epidermidis isolates, which also showed a high diversity in their PFGE patterns. A clone was found that amplified both SCCmec III and V elements in five isolates examined. The single MR S. pettenkoferi isolate harboured SCCmec type IVd and the single MR S. pasteuri isolate harboured SCCmec type I. The carriage of SCCmec type III was associated with resistance or intermediate resistance to meropenem (P < 0.05). These results confirm the high prevalence of S. epidermidis SCCmec IVa and the high genetic diversity among MR-CoNS strains. As far as is known, this is the first report describing the newly identified S. pettenkoferi possessing SCCmec IVd and S. pasteuri harbouring SCCmec type I. MR-CoNS harbouring SCCmec type III were found to be more resistant to meropenem.

INTRODUCTION

In recent years, coagulase-negative staphylococci (CoNS) have emerged as important causative agents of illness, frequently involved in nosocomial infections related to medical devices and prostheses in immunocompromised patients (Curtis & Shetty, 2008; Falcone et al., 2007; Viale & Stefani, 2006). This scenario has worsened because of drug-resistant CoNS, such as meticillin-resistant (MR)-CoNS (Lyytikäinen et al., 2002).

Meticillin resistance in staphylococci is conferred by the penicillin-binding protein PBP2a (PBP2a') encoded by the mecA gene and located on the staphylococcal cassette chromosome mec (SCCmec) (Katayama et al., 2000). Eight different SCCmec types (I–VIII) have been identified and

Abbreviations: CoNS, coagulase-negative staphylococci; MR, meticillin-resistant; MRSA, meticillin-resistant Staphylococcus aureus; MRSE, meticillin-resistant Staphylococcus epidermidis; SCCmec, staphylococcal cassette chromosome mec.

The GenBank/EMBL/DDBJ accession numbers for the partial DNA sequences of the 16S rRNA gene and SCCmec type IVd of Staphylococcus pettenkoferi are GQ145596 and GQ145595, respectively.
Table 1. Distribution of SCCmec cassettes among MR staphylococcal isolates

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Species</th>
<th>Patient age (years)</th>
<th>Patient gender (male/female)</th>
<th>Specimen source</th>
<th>ccr</th>
<th>mec complex</th>
<th>SCCmec type</th>
<th>MIC (µg ml⁻¹)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1492</td>
<td>S. epidermidis</td>
<td>17 M</td>
<td>Catheter</td>
<td>NT</td>
<td>A</td>
<td>III</td>
<td>AMX &gt;8/4</td>
<td>TZP &gt;16/4</td>
</tr>
<tr>
<td>1237</td>
<td>S. epidermidis</td>
<td>78 M</td>
<td>Bone</td>
<td>NT</td>
<td>NT</td>
<td>III and IVa</td>
<td>AMX &gt;16/4</td>
<td>TZP &gt;16/4</td>
</tr>
<tr>
<td>2390</td>
<td>S. epidermidis</td>
<td>0 F</td>
<td>Catheter</td>
<td>NT</td>
<td>A+B</td>
<td>III and IVa</td>
<td>LVX &gt;16/4</td>
<td>CRO &gt;16/4</td>
</tr>
<tr>
<td>1337</td>
<td>S. epidermidis</td>
<td>35 M</td>
<td>Blood</td>
<td>NT</td>
<td>B</td>
<td>III and IVa</td>
<td>AMP &gt;16/4</td>
<td>MIN &gt;16/4</td>
</tr>
</tbody>
</table>

*For each isolate, the MIC values are given for various antibiotics, with concentrations ranging from 0.06 to 16 µg ml⁻¹.
Table 1. cont.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Species</th>
<th>Patient age (years)</th>
<th>Patient gender (male/female)</th>
<th>Specimen source</th>
<th>ccr</th>
<th>mec complex</th>
<th>SCCmec type</th>
<th>MIC (µg ml⁻¹)*</th>
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</thead>
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<tr>
<td>2452</td>
<td><em>S. haemolyticus</em></td>
<td>7</td>
<td>M</td>
<td>Blood</td>
<td>1</td>
<td>NT</td>
<td>NT</td>
<td>&gt;8/4 &gt;16/4 &gt;32 &gt;64 &gt;16 0.5 0.25 &gt;16</td>
</tr>
<tr>
<td>663</td>
<td><em>S. hominis</em></td>
<td>27</td>
<td>M</td>
<td>Bone</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>0.25/0.12 &gt;16/4 2 2 4 0.5 0.125 0.12</td>
</tr>
<tr>
<td>1494</td>
<td><em>S. hominis</em></td>
<td>36</td>
<td>M</td>
<td>Bone</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>0.25/0.12 1/4 8 4 &gt;16 &gt;8 2 1</td>
</tr>
<tr>
<td>397</td>
<td><em>S. hominis</em></td>
<td>0</td>
<td>F</td>
<td>Blood</td>
<td>1 and 5</td>
<td>A</td>
<td>III</td>
<td>&gt;8/4 &gt;16/4 &gt;32 32 16 &gt;8 2 &gt;16</td>
</tr>
<tr>
<td>57</td>
<td><em>S. cohni</em></td>
<td>NA</td>
<td>M</td>
<td>Blood</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>&gt;8/4 &gt;16/4 &gt;32 &gt;64 &gt;16 &gt;8 &gt;16 &gt;16</td>
</tr>
<tr>
<td>2074</td>
<td><em>S. cohni</em></td>
<td>52</td>
<td>F</td>
<td>Catheter</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>&gt;8/4 8/4 &gt;32 &gt;64 8 &lt;0.25 0.5 2</td>
</tr>
<tr>
<td>349</td>
<td><em>S. cohni</em></td>
<td>53</td>
<td>M</td>
<td>Catheter</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>8/4 &gt;16/4 &gt;32 &gt;64 4 &gt;8 2 2</td>
</tr>
<tr>
<td>3281</td>
<td><em>S. cohni</em></td>
<td>0</td>
<td>F</td>
<td>Blood</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>2/1 8/4 0.25 &gt;64 1 &gt;8 0.125 0.25</td>
</tr>
<tr>
<td>849</td>
<td><em>S. cohni</em></td>
<td>20</td>
<td>F</td>
<td>Catheter</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>4/2 8/4 &gt;32 32 1 1 0.25 0.5</td>
</tr>
<tr>
<td>2658</td>
<td><em>S. pettenkoferi</em></td>
<td>0</td>
<td>M</td>
<td>Blood</td>
<td>NT</td>
<td>NT</td>
<td>IVd</td>
<td>&gt;8/4 &gt;16/4 &gt;32 &gt;64 16 &lt;0.25 0.25 &gt;16</td>
</tr>
<tr>
<td>2247</td>
<td><em>S. sciuri</em></td>
<td>26</td>
<td>M</td>
<td>Bone</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>4/2 16/4 0.5 32 2 &lt;0.25 0.25 1</td>
</tr>
<tr>
<td>3223</td>
<td><em>S. pasteuri</em></td>
<td>0</td>
<td>F</td>
<td>Bone</td>
<td>NT</td>
<td>NT</td>
<td>1</td>
<td>≤2 8/4 4 4 &gt;8 &lt;0.25 0.25 1</td>
</tr>
</tbody>
</table>

NA, Data not available; NT, non-typable.

*AMX, amoxicillin/clavulanic acid; TZP, piperacillin/tazobactam; LVX, levofloxacin; CRO, ceftriaxone; AMP, ampicillin; MIN, minocycline; TIG, tigecycline; MEM, meropenem.
are categorized by different sets of chromosome cassette recombinase (ccr) genes and differences in the mec gene complex (Chongtrakool et al., 2006; Ito et al., 2001; Ma et al., 2002; Zhang et al., 2009). Although the mec origin remains unknown, it has been suggested that SCCmec can be transferred between staphylococci and that mecA-positive CoNS may act as potential SCCmec donors, accounting for the rise in new MR Staphylococcus aureus (MRSA) clones (Hanssen & Ericson Sollid, 2006; Musser & Kapur, 1992). In relation to the origin of the SCCmec complex, it has been suggested that Macroccocus caseolyticus may be closely associated with the origin of the meticillin resistance gene complex in S. aureus (Baba et al., 2002).

The objective of this study was to determine the distribution of SCCmec elements in MR-CoNS isolated from a tertiary-care hospital in Mexico and to examine its relationship to drug resistance.

**RESULTS AND DISCUSSION**

**Staphylococci identified**

We evaluated the distribution of SCCmec types in CoNS isolated from patients at the Hospital Universitario Dr José Eleuterio Gonzalez over a 6-month period. From 215 clinical isolates included in the study, 149 were MR-CoNS and we randomly selected 50 isolates for further studies. Among the 50 selected MR-CoNS clinical isolates, as reported by others in similar studies, S. epidermidis was the most common species identified (n=26) followed by Staphylococcus haemolyticus (n=13) (Mombach Pinheiro Machado et al., 2007; Ruppé et al., 2009) (Table 1). Species identification of 17 of the isolates, including some Staphylococcus haemolyticus, and all Staphylococcus cohnii, Staphylococcus hominis and the single Staphylococcus sciuri, Staphylococcus pasteurii and Staphylococcus pettenkoferi isolates was confirmed by PCR and partial sequencing of the 16S RNA gene. The fragments obtained were compared with GenBank sequence entries using the BLAST algorithm (http://www.ncbi.nlm.nih.gov/BLAST).

For the recently described S. pettenkoferi, the sequence gave 99% identity with S. pettenkoferi strains SNUBH406 (GenBank accession no. FJ222447.1), TPL06 (EU373375.1), K6999 (AM266221.1), 229 (DQ538517.1), 230 (DQ538518.1) and 231 (DQ538519.1) and the A6664 (DQ538520) strain previously described by Trüllszch et al. (2002, 2007). Using the API Staph V4.0 identification system (bioMérieux), this strain was originally identified as Kocuria varians with an identification probability of 94.4%.

The single S. pasteuri isolate was identified as S. aureus using the API Staph V4.0 identification system, with an identification probability of 96.9%. The coagulase result was confirmed as negative. For the S. pasteuri isolate, the sequence gave 100% identity with S. pasteuri strains D4027 (FJ161258.1), JS10 (FJ205745.1) and NQ20 (EU919211.1).

**SCCmec types identified**

Among the MR S. epidermidis (MRSE) isolates examined, three harboured SCCmec type III, five had both III and IVa elements and eight harboured SCCmec type IVa (Table 1). The most frequent SCCmec element for S. epidermidis or any other species examined was IVa, which was recently described as the predominant type among S. epidermidis clinical isolates by Jamaluddin et al. (2008) in a Japanese population. In addition, in a long-term care facility in...
Finland, the distribution of SCCmec types in *S. epidermidis* was diverse, with the majority belonging to type IV (33%), followed by type V (18%) (Ibrahem et al., 2009). A report that included 44 strains isolated between 1973 and 1983 from blood showed that 16 strains (36%) harboured SCCmec type IV (Wisplinghoff et al., 2003). In addition, Ruppé et al. (2009) described 75 MRSE strains from four countries, 34 of which were non-typable for SCCmec. Among the typable strains, type IV was the most common (27/75), followed by type V (13/75) (Ruppé et al., 2009). In contrast, Mombach Pinheiro Machado et al. (2007) found only 1/87 MRSE strains harbouring SCCmec type IV, with the most frequent MRSE SCCmec type being type III–IV (30/87). In our study, only 15% of *S. epidermidis* strains were untypable for SCCmec. Furthermore, the isolation of strains that amplified two types of SCCmec [III and IVa (five isolates), III and V (six isolates), and II and V (four isolates)] strongly suggests that new variants may be present in CoNS and may have a different impact on drug resistance. The typing of isolates that amplified two SCCmec types was confirmed by using single primers for each locus.

Evidence has suggested horizontal transfer of SCCmec from MR *S. haemolyticus* to meticillin-susceptible *S. aureus* strains, resulting in the creation of a new MRSA clone that could result in a potential outbreak (Berglund & Söderquist, 2008). This concern underlines the importance of studying the prevalence of SCCmec in all CoNS strains.

In this study, we obtained only one isolate each of *S. sciuri*, *S. pasteuri* and *S. pettenkoferi*. The MR *S. pettenkoferi* isolate harboured SCCmec type IVd. An alignment of the DNA sequence of type IVd SCCmec of *S. pettenkoferi* and *S. aureus* type IV.4 (IVd) (GenBank accession no. AB097677.1) showed 100% sequence identity (identities = 848/848), as did the alignment with *S. aureus* type IVd cassette chromosome mec (GenBank accession no. EF634484.1) (identities = 746/746). To our knowledge, this is the first description of the presence of SCCmec in *S. pasteuri* and of the isolation of this species from bone.

The single *S. pasteuri* isolate was isolated from bone in a newborn female with trisomy 21 and interauricular and interventricular communication, who had had multiple transfusions of blood and platelets. Interestingly, this particular species has been isolated from a leukemic patient with bacteraemia (Savini et al., 2009a) and as a contaminant of platelet units (Savini et al., 2008, 2009b). To our knowledge, this is the first description of the isolation of this species from bone.

The single MR *S. pasteuri* isolate harboured SCCmec type I. The alignment of type I SCCmec of *S. pasteuri* and *S. aureus* NCTC 10442 showed 100% sequence identity.

**PFGE genotyping and genotype cluster distribution**

Evaluation of banding patterns revealed an *S. epidermidis* clone with 100% homology in the PFGE patterns that amplified for both SCCmec III and V in samples 965, 1439, 1543, 1955 and 1981 (Fig. 1). This clone was isolated over a period slightly longer than 3 months (30 June 2006 to 4 October 2006). It is not likely that this clone could transmit these genetic elements to *S. aureus* isolates because it has been shown that the type 3 *ccr* has lost the function of excising and integrating SCCmec into the staphylococcal chromosome. Instead, MRSE carrying SCCmec type IV is probably a reservoir of SCCmec that can be transferred to other species. The most frequent species and genotype of MR-CoNS isolated in our hospital was *S. epidermidis* SCCmec type IVa and these isolates showed high diversity. In Fig. 1, we have shown the only isolates with the same

![Fig. 1. PFGE dendrogram comparing representative *S. epidermidis* clones from the Hospital Universitario Dr José Eleuterio Gonzalez. The SCCmec type is included. The bar represents similarity. Similarity coefficients were generated from a similarity matrix calculated with the Jaccard coefficient using SPSS 10.0 software.](http://jmm.sgmjournals.org)
PFGE pattern and some examples of the diversity found in the SCCmec IVa. All other PFGE patterns were completely different and no major cluster was detected among these strains.

**Antibiotic susceptibility testing**

The results of susceptibility testing are shown in Table 1. In this study, we detected the previously described high resistance to antibiotics associated with the presence of SCCmec type III (Ito et al., 2001), although the only statistically significant association found was the carriage of SCCmec type III with resistance or intermediate resistance to meropenem \((P < 0.05)\). Interestingly, three out of four strains that amplified only SCCmec III were resistant to tigecycline and the strains that amplified SCCmec type III plus another type (III and IVa, or III and V) were all susceptible to this drug.

In conclusion, we confirmed the high genetic diversity among the MR-CoNS isolates studied. This is the first report describing *S. pettenkoferi* and *S. pasteurii* carrying SCCmec elements and the first description of an *S. epidermidis* clone that amplified both SCCmec type III and V elements. These data are important not only for defining hospital control strategies, but also for shedding light on the heterogeneous pool of SCCmec types present in CoNS strains, which may act as a source of virulence factors for more pathogenic *S. aureus* strains.

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**REFERENCES**


