Carriage of Methicillin-Resistant Staphylococci and Their SCCmec Types in a Long-Term-Care Facility

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Following an outbreak caused by staphylococcal cassette chromosome mec (SCCmec) type V methicillin (meticillin)-resistant Staphylococcus aureus (MRSA), a point-prevalence survey of the nasal carriage of staphylococci was conducted in a long-term-care facility in northern Finland in 2004. The focus was directed at methicillin-resistant coagulase-negative staphylococci (MR-CNS) and their SCCmec elements. A nasal swab was taken from 76 of the 80 residents 6 months after the onset of the outbreak. Staphylococcal isolates were identified by conventional methods and the GenoType Staphylococcus test, and their SCCmec elements were analyzed. Of the 76 individuals, 24 (32%) carried S. aureus and 67 (88%) CNS in their nostrils. Of the CNS carriers, 41 (61%) had at least one mecA-positive MR-CNS, and two individuals (3%) had both MRSA and methicillin-resistant Staphylococcus epidermidis (MRSE). Among the 61 MR-CNS isolates identified, 49 (80%) were MRSE. The distribution of the SCCmec types was diverse: 20 (33%) were of type IV, 11 (18%) of type V, 4 (6%) of type I or IA, 3 (4%) of type II, and 23 (38%) of new types (with six different combinations of ccr and other mec genes or only mecA). Both of the individuals with MRSA and MRSE shared SCCmec type V among their isolates. Nasal MR-CNS carriage was common among the residents of this long-term-care facility. A variety of SCCmec types, including many new types, were identified among the MR-CNS strains. The horizontal transfer of SCCmec elements is speculated based on the sharing of SCCmec type V between MRSA and MRSE.

Coagulase-negative staphylococci (CNS) belong to the normal microbial flora of the skin and mucous membranes of humans. The most frequently encountered CNS species in humans, in decreasing order of occurrence, are Staphylococcus epidermidis, Staphylococcus haemolyticus, Staphylococcus saprophyticus, and Staphylococcus lugdunensis (8). CNS are an important cause of nosocomial infections, particularly causing foreign device-related infections and infections among immunocompromised patients. In a recent prospective laboratory-based surveillance in four Finnish acute-care hospitals, 76% of the blood culture CNS isolates were resistant to methicillin (meticillin) (23).

Methicillin resistance in staphylococci is caused by the expression of penicillin-binding protein PBP2a (PBPs), which is encoded by the mecA gene. In S. aureus and CNS, mecA is located on a genetic element called the staphylococcal cassette chromosome (SCCmec) (15, 37). SCCmec is integrated into the chromosome of S. aureus at a unique site (attBcsc) located near the S. aureus origin of replication. Up to now, six different SCCmec types (I to VI) have been recognized, each of which is different in size (21 to 67 kb) and characterized by a different set of ccr recombinase genes and mec gene complex (3, 12, 13, 22, 24, 32). In addition to the major types, a number of new SCCmec elements, including non-mecA-encoding cassettes, have been discovered (2, 3, 11, 16, 19, 20, 28). New types may be generated continuously (5, 9).

The SCCmec has been identified exclusively among staphylococci, but its origin remains unknown (9). It has been suggested that the ccr and mec genes from an unknown source were brought together in CNS (34, 38), and a deletion in the mec regulatory genes occurred before the cassette was transferred into S. aureus to create methicillin-resistant S. aureus (MRSA) (10, 30). The transfer of mecA from S. epidermidis to S. aureus has been suspected to occur in vivo (36). However, the mechanisms responsible for the possible horizontal transfer of mecA between staphylococcal species or between different gram-positive species are not known. Evaluations of the epidemiology of methicillin-resistant staphylococcal colonization and SCCmec typing are necessary to understand the apparent emergence of MRSA strains from CNS.

This point-prevalence study of the nasal carriage of CNS was conducted 6 months after an outbreak of MRSA in a long-term-care facility (LTF) in northern Finland in 2004. The MRSA outbreak was caused by a strain that had not been encountered previously in Finland, FIN-22, with SCCmec type V (17). In this study, we focused on the structure of the SCCmec elements of methicillin-resistant (MR)-CNS strains and a structural comparison of SCCmec elements of methicillin-resistant S. epidermidis (MRSE) and MRSA isolated from the same person at the same time, under the suspicion of horizontal SCCmec transfer in vivo.

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TABLE 1. Number of persons with different combinations of MRSA and methicillin-susceptible S. aureus (MSSA) and CNS

<table>
<thead>
<tr>
<th>Strain</th>
<th>No. of MRSA isolates</th>
<th>No. of MSSA isolates</th>
<th>No. of CNS isolates</th>
<th>Total (n = 73)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MR-CNS</td>
<td>2</td>
<td>11</td>
<td>28</td>
<td>41</td>
</tr>
<tr>
<td>MS-CNS</td>
<td>1</td>
<td>4</td>
<td>21</td>
<td>26</td>
</tr>
<tr>
<td>S. aureus</td>
<td>2</td>
<td>4</td>
<td>6</td>
<td></td>
</tr>
</tbody>
</table>

* MS, methicillin susceptible.

FIG. 1. SCCmec multiplex patterns of MR-CNS strains (31). M, molecular size markers (in kilodaltons). Lanes 1 to 4 and 21 to 24, control MRSA of type IA (locus A, upstream of the pI258 region); locus B, 110 bp. Lanes 5 to 20, MRSE isolates; lanes 25 to 31 and 33, methicillin-resistant S. capitis; lane 32, methicillin-resistant S. hominis; and lane 34, methicillin-resistant S. hominis.

RESULTS

Of the 76 patients, 73 (96%) were colonized with a staphylococcal species (Table 1): 67 (92%) were colonized by at least one CNS strain, 49 (73%) by CNS only, and 18 (27%) by CNS in combination with S. aureus. S. aureus alone was found in six persons (8%). Of 67 CNS carriers, 41 (61%) were colonized with at least one MR-CNS strain, and two of them carried MRSA as well. Twenty-six (39%) patients were colonized by methicillin-susceptible CNS strains, and one of them carried MRSA as well.

From the 67 patients with CNS, 127 isolates were obtained. The number of isolates per individual varied from one to five. Of the 127 CNS isolates, 61 (48%) were shown to be methicillin resistant. These included 49 (80%) of 82 having S. epidermidis isolates, 10 (66%) of 15 having S. capitis isolates, 1 (14%) of 7 having S. haemolyticus isolates, and 1 (50%) of 2 having S. hominis isolates.

Among the 49 MRSE isolates, three MRSE strains could be...
classified as SCCmec type I (Fig. 1, lane 5) and two as SCCmec type IV (Fig. 1, lane 7); in one MRSE isolate, only mecA was amplified (Fig. 1, lane 15). The remaining 43 MRSE isolates could not be interpreted as belonging to any of the currently described SCCmec types (31). Within the other MR-CNS species, one methicillin-resistant S. capitis isolate could be classified as SCCmec type IA (Fig. 1, lane 25), while seven methicillin-resistant S. capitis isolates and one methicillin-resistant S. haemolyticus isolate (Fig. 1, lane 32) could not be recognized as belonging to any of the currently known SCCmec type. In two methicillin-resistant S. capitis isolates and one methicillin-resistant S. hominis isolate, only mecA was amplified (Table 2 and Fig. 1, lanes 30, 31, and 34).

By analyzing the ccr and mec components (19), the MRSE isolates could be categorized as follows: 3 (6%) harbored SCCmec type I, 2 (4%) type II, 18 (37%) type IV, and 9 (12%) type V. Three isolates were of a nontypeable SCCmec type, as neither ccr genes nor mec genes could be amplified. The remaining 14 isolates had ccr and mec complex gene combinations for which no names have been assigned previously (Table 2). Among the 10 methicillin-resistant S. capitis isolates, three harbored SCCmec types IA, II, and IV, and two harbored type V. The remaining five isolates harbored a new SCCmec type (Table 2). The single methicillin-resistant S. haemolyticus isolate harbored SCCmec type IV, and the methicillin-resistant S. hominis isolate harbored a new SCCmec type (Table 2).

Two patients were colonized by both MRSE and MRSA. The first one carried an MRSA strain and three MRSE strains. The MRSA strain and one of the MRSE strains harbored SCCmec type V (ccrC and mec class A), while the two MRSE strains were of different SCCmec types, type II (ccrA2 and class A) and a new SCCmec type (ccrC and mec, class A). The second carrier had MRSA and MRSE, both of which harbored SCCmec type V (Fig. 2B and C and 3). The MRSE multiplex SCCmec patterns varied among the isolates (Fig. 2A). Genotyping by PFGE revealed that the two MRSA strains were representatives of a Finnish epidemic strain (FIN-7 and FIN-7 subtype), and the four MRSE strains had unique PFGE profiles (Fig. 3).

**DISCUSSION**

The nasal carriage of MR-CNS was found to be common among the residents of the studied LTF. The MRSE isolate was the most prevalent CNS species. A diversity of SCCmec types, with many new combinations of elements as well as nontypeable types, were recognized among the MR-CNS strains. The horizontal transfer of SCCmec elements is speculated based on the sharing of SCCmec type V between MRSA and MRSE in two patients.

The prevalence of CNS carriage among patients participating in this study was high, at 92%. The proportion of the nasal carriage of MR-CNS among patients in an LTF in this study was somewhat higher (48%) than that in a similar study in the United States (40%) (21). However, there is a very limited number of reports on MR-CNS nasal carriage among patients in long-term-care settings. Consistently with one such previous report (33), the most common MR-CNS species in our study was MRSE.

The SCCmec typing of MR-CNS isolates revealed that 62%
of the isolates harbored previously recognized SCCmec types (I, IA, II, and IV). For the remaining 37%, ccr and mec complexes could not be amplified at all, or a variety of new combinations was detected. One-third of the MR-CNS isolates had SCCmec type IV, and SCCmec type IV was most prevalent among the S. epidermidis strains (37%). Among the 20 strains, 18 harbored a modified SCCmec type IV. While the originally described type IV contains only locus D (dcs region), we identified several additional loci amplified from type IV strains in different combinations (Fig. 1, lanes 8 to 10 and 32, and Table 2). The combinations were not species specific. Modified patterns also were found among other SCCmec types; SCCmec type V was found among 11 MR-CNS strains, which represented four different multiplex SCCmec patterns (Fig. 1 and

![PFGE dendrogram of the methicillin-resistant staphylococcal isolates from two patients colonized with MRSA and MRSE.](http://jcm.asm.org/)
Table 2). Three MR-CNS strains harboring SCCmec type II did not have locus B (kdp operon). The remaining 23 strains harbored a new SCCmec type; these strains carried either (i) known SCCmec types with additional elements (i.e., type IV and ccrC or type V and ccrA) or (ii) combinations of ccr and mec that could not be interpreted as belonging to any of the presently described SCCmec types (Table 2). Previous studies also have shown variations in SCCmec cassettes. These include (i) strains containing both SCCmec type IV and ccrC, (ii) strains carrying multiple ccr genes (3, 7, 14, 26), (iii) strains carrying ccr genes without a mec complex, (iv) strains carrying a mec complex without ccr genes, and (v) a mecA-positive MRSA strain with neither ccr genes nor a mec complex (3, 25, 26). In our study, only mecA from three MR-CNS strains could be detected. The failure to amplify ccr and mec may indicate that the target sequences for primers have changed.

Defining SCCmec types in MR-CNS strains based solely on amplifying sequences between and flanking the ccr genes and the mec complex raises some concerns (26). These areas do not contain specific loci for a specific SCCmec type. For instance, locus A was previously thought to be part of SCCmec type I and IA only, but according to this study, it also is present in types II, IV, and V. Moreover, the SCCmec types IV and V contained a variety of loci. Locus B previously has been defined to be specific for SCCmec type II, but we recognized locus B as being present in type V and in three new types. Therefore, the detection of these intervening sequences provides valuable additional information on the discrimination of SCCmec types.

We have previously reported on the MRSA nasal carriage of this study population (17). In total, five different MRSA strains were identified, and all of them had SCCmec type V. In the present study, we analyzed in detail the two patients who carried both MRSE and MRSA strains simultaneously, and all of these isolates shared SCCmec type V (ccrC, class C). However, differences in the J-region sequences were identified between MRSA and MRSE strains (additional loci E and A in MRSE) (Fig. 2A and 3). Although we are not able to rule out the possibility that the similar SCCmec cassettes were acquired through different routes, this observation supports the possibility of SCCmec transfer. If such a transfer has happened, it was not reported. Further studies revealing the mechanisms of SCCmec transfer are needed. The hypothesis for the transfer of SCCmec between S. epidermidis and S. aureus has been previously reported (6, 7, 27, 37).

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REFERENCES


