Molecular characterization of methicillin-resistant *Staphylococcus epidermidis* strains from bacteraemic patients

S. Ibrahem¹, S. Salmenlinna¹ ², O. Lyytikäinen³, M. Vaara⁴ and J. Vuopio-Varkila¹

¹Department of Bacterial and Inflammatory Diseases, National Public Health Institute, ²Department of Bacteriology and Immunology, Haartman Institute, ³Department of Infectious Disease Epidemiology, National Public Health Institute and ⁴Division of Clinical Microbiology, Helsinki University Central Hospital Laboratory, Helsinki, Finland

**ABSTRACT**

In order to study the clonality of clinical methicillin-resistant *Staphylococcus epidermidis* (MRSE) strains and their staphylococcal cassette chromosome mec (SCCmec) elements, 60 isolates of MRSE from bacteraemic patients in three units of the Helsinki University Hospital, Finland were selected, covering the periods 1990–1993 and 1997–1998. The MRSE strains were analysed by pulsed-field gel electrophoresis (PFGE), multilocus sequence typing and SCCmec typing. Eleven PFGE types (FIN-SE-1–11) with sequence type ST2 (clonal complex 2; CC2) were identified. The previously established methicillin-resistant *Staphylococcus aureus* SCCmec criteria were applied to name the MRSE SCCmec complexes, and it was found that 7% of the isolates carried SCCmec type IA (*ccrA1*, class B), whereas the majority (93%) yielded six non-typeable SCCmec PCR patterns (P1–P6). Within each SCCmec PCR pattern, two ccr recombinase genes (*ccrA2* and *ccrA3*) and two mec gene complexes (class A and class B) were detected. In addition, the *ccrC* gene was associated with three of the six patterns. In conclusion, the MRSE strains were genetically related to each other (ST2) but their SCCmec complexes were unique combinations of elements previously recognized among SCCmec types III and IV.

**Keywords**  Bacteraemic strains, complex SCCmec, molecular typing, MRSE, persistent clone

**Original Submission:** 2 July 2007;  **Revised Submission:** 28 March 2008;  **Accepted:** 31 March 2008

Edited by D. Mack

*Clin Microbiol Infect* 2008; 14: 1020–1027
previously [3,6–8]: types I (ccrA1, class B), II (ccrA2, class A), III (ccrA3, class A), IV (ccrA2, class B) and V (ccrC, class C). In addition, SCCmec type VI (ccrA4, class B) has been identified as a structural variant of type IV [9]. The variants of each SCCmec type are defined by differences in the J regions.

The nomenclature of SCCmec types has been created mostly on the basis of *Staphylococcus aureus* SCCmec structures. There are some studies on the composition of the SCCmec elements in other staphylococcal species [10–12]. In the early 1970s, SCCmec type IV was prevalent in *S. epidermidis* isolates [13]. Recently, numerous structural types of SCCmec, including types I–V, have been observed in MRSE by Miragaia et al. [1,14], and Hanssen et al. [15] suggested that further diversity arises when a single MRSE strain carries multiple SCCmec types.

In this study, molecular typing techniques were used to characterize a set of clinical MRSE isolates in Finland. The focus was both the analysis of clonality of the isolates and the characterization of their SCCmec elements. The previously recognized methicillin-resistant *S. aureus* (MRSA) SCCmec typing techniques and criteria were used to name the MRSE SCCmec complexes [3,6,16], and it was found that the majority of them remained unrecognizable or were new combination types. Using this information in combination with the pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST) data, it was also possible to show that certain *S. epidermidis* strains persist in the units for a long period of time.

**MATERIALS AND METHODS**

**Bacterial isolates**

Sixty clinical MRSE blood isolates from three collections were included. The first comprised 23 isolates from a neonatal intensive-care unit (NICU) and four isolates from a paediatric haematology unit (PHU) of the Children’s Hospital of Helsinki University Central Hospital (HUCH) collected between October 1990 and August 1992. The second collection comprised 11 isolates from two adult haematology units (AHU-A and AHU-B) of HUCH collected between October 1992 and February 1993. These isolates were recognized as true bacteremia-causing isolates on the basis of previously published clinical criteria [17,18]. They have been characterized by three typing techniques (antibiogram, plasmid profile and ribotyping), and were considered to represent the most prevalent strains causing MRSE bacteremia within those hospital units [17,18]. The third collection comprised 22 consecutive blood isolates cultured from the above-mentioned NICU between March 1997 and May 1998.

**SCCmec typing**

The initial classification of the SCCmec types was performed using multiplex PCR as previously described [16], with slight modifications. Briefly, genomic DNA was prepared by the rapid extraction of bacterial genomic DNA with a guanidinium thiocyanate method [19].

A 50-μL PCR mixture consisted of 10× PCR buffer II, 200 μM (each) deoxyribonucleotide triphosphate, 1.8 mM MgCl₂, 2.5 U of AmpliTag DNA polymerase (Applied Biosystems, Foster City, CA, USA), and 15 ng of template DNA. The primer concentrations were the same as previously described [15]. The sequences are listed in Table 1. The PCR products (8 μL) were resolved in a Sea Kem agarose (2%) gel (FMC BioProducts, Rockland, ME, USA) in 0.5× TBE buffer at 100 V and visualized with ethidium bromide. The pUC Mix Marker 8 (Fermentas Life Sciences, Burlington, Ontario, Canada) was used as a molecular weight standard.

To check for the existence of individual genes and other DNA areas within the SCCmec element, the short-range and long-range PCRs were used as previously described [6,8,20]. The amplified products were resolved in Sea Kem agarose (0.7%) gel and stained with ethidium bromide. The DNA molecular weight markers XVII (0.5–5 kb) and II (0.5–23 kb) (Roche Diagnostics GmbH, Mannheim, Germany) were used as migration standards.

The oligonucleotide primers (Sigma-Genosys, Cambridge, UK) used in the PCR assays are listed in Table 1. The following MRSA strains were used as positive controls for SCCmec typing: the Iberian clone (HPV107, type IA, ccrA1 and class B); UK EMRSA-16 (96/32010, type II, ccrA2 and class A); the Brazilian clone (HS216, type IIIA, ccrA3 and class A) [16], the Paediatric clone (HDE288, type VI, ccrA4 and class B) [9]; and a Finnish EMRSA (FIN-22, type V, ccrC and class C) [21].

**PFGE**

All isolates were genotyped using PFGE. The genomic DNA was prepared in agarose blocks according to a rapid DNA preparation procedure [22]. Running conditions were according to the Harmony protocol [23]. PFGE patterns were analysed with BIONUMERICS (version 2.0; Applied Maths, Kortrijk, Belgium). After initial classification by computer-assisted analysis, the patterns were further interpreted according to established guidelines [24]. PFGE patterns with seven or more band differences were considered to represent different types, and those with one to six band differences to represent subtypes. PFGE types were assigned a FIN-SE number, and subtypes were assigned letters.

**MLST**

Twelve strains (representing 11 different PFGE types and one subtype) and one reference strain (*S. epidermidis* strain ATCC 12228) were analysed using MLST [25,26] with the primers of Thomas et al. [26] (Table 2). For sequence analysis, the Vector NTI program (Invivogen Corporation, Carlsbad, CA, USA) was used. The alleles and the allelic profiles of each of the seven housekeeping loci (*arcC, arcE*, *gtr, mutS, pyrK, tpi, yqiL*) and the sequence types (STs) were obtained from the MLST database (http://s.epidermidis.mlst.net).
FIN-SE-1 15 NICU, PHU, AHU-B, of the pfl gene
Locus B, kdp operon
Locus C, mec gene
Locus D, des region
Locus E, between integrated
p2538 and Tn554
Locus F, between Tn554 and orfX
Locus G, left junction between
IS431 and pUB110
Locus H, left junction between
IS431 and pT181
crr typing
ccrA3
ccrB
ccrC
ccrA2–ccrA3
mec typing
IS431–mecA
ISB172–mecA
mcr–mecA
des region–mecA
ISB172–mecR (MS)
ISB172–mecR (PB)
mecR (PB) domain
mecI
mec operon
TnpA (Tn554)

Table 1. Primers used in SCCmec typing

<table>
<thead>
<tr>
<th>Target element</th>
<th>Forward primer (sequence 5’ → 3’)</th>
<th>Reverse primer (sequence 5’ → 3’)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multiplex PCR typing mecA, internal control</td>
<td>MECAH (TCTAGATCTACACTATACGG)</td>
<td>MECAH (TACCTACATCATTGATCTG)</td>
<td>[16]</td>
</tr>
<tr>
<td>Locus A, upstream of the pfl gene</td>
<td>CIEF2 (TCTGACCTGCTGATGAA)</td>
<td>CIEF2 (ATTACACCAAGGCACCTGC)</td>
<td>[16]</td>
</tr>
<tr>
<td>Locus B, kdp operon</td>
<td>KDF F1 (AATACCTGCGCTGATTTG)</td>
<td>KDF F1 (CAAATAGCTAGGAAAGATG)</td>
<td>[16]</td>
</tr>
<tr>
<td>Locus C, mec gene</td>
<td>MECAH P3 (ATCAGAAGTCTTATATATG)</td>
<td>MECAH P3 (GCGGTTTCACCGAGCGCA)</td>
<td>[16]</td>
</tr>
<tr>
<td>Locus D, des region</td>
<td>DCS F2 (CATCTTCTGATATTGCTTG)</td>
<td>DCS F2 (TTCGAGTTGCTGATGAG)</td>
<td>[16]</td>
</tr>
<tr>
<td>Locus E, between integrated p2538 and Tn554</td>
<td>RIF F3 (GCTTGGTTCGATACATACG)</td>
<td>RIF F3 (GCTTTTATCGGTATGATG)</td>
<td>[16]</td>
</tr>
<tr>
<td>Locus F, between Tn554 and orfX</td>
<td>RIF F10 (TTCCTGATAGCTGATTTTG)</td>
<td>RIF F10 (GTCACTATATGCTGATGATG)</td>
<td>[16]</td>
</tr>
<tr>
<td>Locus G, left junction between IS431 and pUB110</td>
<td>IS431 P4 (CAGGGCTCCATGCTTACG)</td>
<td>IS431 P4 (GAAGGCATGAAAGACAGAA)</td>
<td>[16]</td>
</tr>
</tbody>
</table>

Table 2. Characteristics of Finnish bacteraemic methicillin-resistant Staphylococcus epidermidis isolates

<table>
<thead>
<tr>
<th>PFGE type</th>
<th>No. of isolates</th>
<th>Hospital units</th>
<th>Year of isolation</th>
<th>Antibiotic resistance profile</th>
<th>SCCmec pattern</th>
<th>MLST profile</th>
<th>ST</th>
</tr>
</thead>
<tbody>
<tr>
<td>FIN-SE-1 15 NICU, PHU, AHU-B</td>
<td>1990–1992</td>
<td>OXA, ERY, CLI, CHL, GEN, TOB</td>
<td>P1: F, G, D, E, C, mecA</td>
<td>7-1-2-4-1-1</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>FIN-SE-3 1 NICU</td>
<td>1993</td>
<td>OXA, ERY, SXT, TOB</td>
<td>P3: G, C, mecA</td>
<td>7-1-2-4-1-1</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>FIN-SE-4 4 AHU-B</td>
<td>1992</td>
<td>OXA, ERY, RIF, FUS, GEN, TOB</td>
<td>S SCCmec type IA</td>
<td>7-1-2-4-1-1</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>FIN-SE-5 2 AHU-A</td>
<td>1992–1993</td>
<td>OXA, ERY, CLI, TET, SXT, GEN, TOB</td>
<td>P4: F, D, E, C, mecA</td>
<td>7-1-2-4-1-1</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>FIN-SE-6 4 PHU and AHU-A</td>
<td>1990, 1993</td>
<td>OXA, ERY, CLI, CIP, TOB</td>
<td>P1: F, G, D, E, C, mecA</td>
<td>7-1-2-4-1-1</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>FIN-SE-7 10 NICU</td>
<td>1997–1998</td>
<td>OXA, ERY, RIF, SXT, GEN, TOB</td>
<td>P5: G, D, C, mecA</td>
<td>7-1-2-4-1-1</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>FIN-SE-8 9 NICU</td>
<td>1997–1998</td>
<td>OXA, ERY, CLI, CLIND, GEN, SXT, TET, trimethoprim-sulphamethoxazole, CIP, ciprofloxacin, TET, tetracycline, CHL, chloramphenicol, FUS, fusidic acid, GEN, gentamicin, TOB, tobramycin, RIF, rifampin.</td>
<td>P5: G, D, C, mecA</td>
<td>7-1-2-4-1-1</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>FIN-SE-9 1 NICU</td>
<td>1997</td>
<td>OXA, ERY, CLI, SXT, FUS, GEN, TOB</td>
<td>P5: D, F, C, mecA</td>
<td>7-1-2-4-1-1</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>FIN-SE-10 1 AHU-A</td>
<td>1991</td>
<td>OXA, ERY, CLI, GEN, TOB</td>
<td>P5: G, D, C, mecA</td>
<td>7-1-2-4-1-1</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>FIN-SE-11 1 AHU-A</td>
<td>1993</td>
<td>OXA, ERY, CLI, SXT, GEN, TOB</td>
<td>P5: D, F, C, mecA</td>
<td>7-1-2-4-1-1</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

RESULTS

SCCmec typing

Multiplex PCR analysis, which reveals eight loci (A–H), in combination with mecA as an internal control [16], yielded amplification patterns in all 60 MRSE strains. Only one of the PCR patterns (found among four strains) could be recognized as a typical S. aureus SCCmec type IA profile (loci A, G and D; Fig. 1, Table 2). The remaining six PCR patterns (found among six strains) did not
amplicons are: (A) 495 bp, (B) 284 bp, (C) 209 bp, (D) 160 bp. M, molecular weight marker.

Fig. 1. SCCmec—multiplex patterns of methicillin-resistant Staphylococcus epidermidis (MRSE). Methicillin-resistant Staphylococcus aureus (MRSA) strains (positive controls): SCCmec type IA (loci A, G and D), SCCmec type II (loci G, D, B and C), SCCmec type IIIA (loci F, E and C), SCCmec type IV (locus D). Internal control: mecA. MRSE strains: P1, IIIA (loci F, E and C) + IVA (loci G and D); P2, IIIB (locus C) + IV (locus D); P3, IIIB (locus C) + IVA (locus G and D); P4, IIIA (loci F, E and C) + IV (locus D); P5, IIIA (locus C) + IVA (loci G and D); P6, IIIA (loci F and C), without locus D; P1–P6 encom- pass of the same molecular weight as that expected for the loci that would represent two different SCCmec types: for P1 IIIA (F, E and C) mec, for P2 IIIA (F, E and C) mecI, and from IS1272 to mecI, and from IS1272 to mecR (PB), and the transposon Tn544 (Tn544), which could not be amplified from DNA yielding any of the SCCmec patterns (P1–P6). The DNA region from the des region to mecA was amplified in the cases of P1, P4, P5 and P6. The mec operon was present in DNA of all six patterns except P3 (Table 3, Fig. 2).

PFGE

Eleven PFGE types (FIN-SE-1 to FIN-SE-11) were assigned among the 60 MRSE isolates (Fig. 3). The number of subtypes within each FIN-SE type varied from zero to six. Only two of the PFGE types could be shown to be shared between strains from different hospital units. The most prevalent PFGE type (FIN-SE-1, n = 18) was found during 1990–1992 in three of the four units (NICU, PHU and AHU-B). FIN-SE-6 was found both in the PHU and AHU-A, but at an interval of 3 years. Several of the strains seemed to be persisting for at least several years (Table 2).

MLST

ST2 was identified among all the 12 different representative PFGE types (including one subtype) studied (Table 2).

DISCUSSION

The purpose of this study was to determine the clonality of 60 clinical MRSE strains and to study their SCCmec elements. The results according to SCCmec typing suggested that the strains may possess elements from two SCCmec types, as is known for MRSA. This assumption was based on several observations. First, P1, P2, P4 and P5 generated by the multiplex PCR showed ampli- cons of the same molecular weight as that expected for the loci that would represent two different SCCmec types: for P1 IIIA (F, E and C) mec, for P2 IIIA (F, E and C) mecI, and from IS1272 to mecI, and from IS1272 to mecR (PB), and the transposon Tn544 (Tn544), which could not be amplified from DNA yielding any of the SCCmec patterns (P1–P6). The DNA region from the des region to mecA was amplified in the cases of P1, P4, P5 and P6. The mec operon was present in DNA of all six patterns except P3 (Table 3, Fig. 2).

PFGE

Eleven PFGE types (FIN-SE-1 to FIN-SE-11) were assigned among the 60 MRSE isolates (Fig. 3). The number of subtypes within each FIN-SE type varied from zero to six. Only two of the PFGE types could be shown to be shared between strains from different hospital units. The most prevalent PFGE type (FIN-SE-1, n = 18) was found during 1990–1992 in three of the four units (NICU, PHU and AHU-B). FIN-SE-6 was found both in the PHU and AHU-A, but at an interval of 3 years. Several of the strains seemed to be persisting for at least several years (Table 2).

MLST

ST2 was identified among all the 12 different representative PFGE types (including one subtype) studied (Table 2).

DISCUSSION

The purpose of this study was to determine the clonality of 60 clinical MRSE strains and to study their SCCmec elements. The results according to SCCmec typing suggested that the strains may possess elements from two SCCmec types, as is known for MRSA. This assumption was based on several observations. First, P1, P2, P4 and P5 generated by the multiplex PCR showed ampli- cons of the same molecular weight as that expected for the loci that would represent two different SCCmec types: for P1 IIIA (F, E and C) mec, for P2 IIIA (F, E and C) mecI, and from IS1272 to mecI, and from IS1272 to mecR (PB), and the transposon Tn544 (Tn544), which could not be amplified from DNA yielding any of the SCCmec patterns (P1–P6). The DNA region from the des region to mecA was amplified in the cases of P1, P4, P5 and P6. The mec operon was present in DNA of all six patterns except P3 (Table 3, Fig. 2).
in MRSA [28,32] and in MRSE [11]. In addition, ccr III lacking TnccrAB3 and P1–P6 encompassed two ccr complexes.

Second, according to the typing of ccr genes corresponding to locus D of SCCmec [27,28]. SCCmec patterns (P1–P6) based on data provided in Tables 1–3. Primers highlighted in red did not yield amplicons. Horizontal arrows within the cassette indicate the direction of SCCmec elements. Loci defined by multiplex PCR are indicated by arrows and appropriate letters (A–G). Unknown regions are indicated by broken lines.

SCCmec P6 showed amplicons corresponding to locus D of SCCmec type IV, whereas loci C and F could correspond to SCCmec type IIIA without locus E. A similar SCCmec type III lacking locus E has been reported previously [28].

Second, according to the typing of ccr genes, P1–P6 encompassed two ccr complexes, ccrAB2 and ccrAB3, but the ccrC gene was found only in P1, P4 and P6. Strains carrying multiple ccr complex genes have been encountered previously [29,30,31]. Third, all patterns encompassed two mec complexes: class A (mecI–mecR1 (MS, PB–mecA) and class B (∆mecR1–mecI). None of the SCCmec patterns, P1–P6, encompassed Tn554. In accordance with this result, an SCCmec III lacking Tn554 has been reported previously in MRSA [28,32] and in MRSE [11]. In addition, the PCR-generated amplicons covering the regions between IS1272 and mecA, between IS1272 and mecR1 (MS) and between mecl and mecA and mecA–mecR1 (PB), and the amplicons were of the expected size (Fig. 2, primers yielding amplicons green) [6,27]. No PCR product was obtained when amplifying the regions between IS1272 and other elements, and the primers highlighted in red did not yield amplicons.

Fig. 2. (a) Schematic structures of methicillin-resistant Staphylococcus aureus (MRSA) SCCmec elements of type III and type IVA based on the findings of Chongtrakool et al. [19] and Oliveira and de Lencastre [15]. (b) Hypothetical structures and arrangements of methicillin-resistant Staphylococcus epidermidis (MRSE) SCCmec patterns (P1–P6) based on data provided in Tables 1–3. Primers highlighted in green yielded the amplified fragment between IS1272 and other elements, and the primers highlighted in red did not yield amplicons. Horizontal arrows within the cassette indicate the direction of SCCmec elements. Loci defined by multiplex PCR are indicated by arrows and appropriate letters (A–G). Unknown regions are indicated by broken lines.
suggesting that the elements from the two plausible SCCmec cassettes could be located close to each other (Fig. 2). The hypothesized arrangement of the P1–P6 SCCmec patterns is in agreement with all PCR results, on the condition that there is an inversion of ccrAB2. Otherwise, the arrangement of two SCCmec cassettes inside the genome remains unexplained. The observed combinations may have resulted from complex recombination and rearrangement processes, generating novel types of SCCmec elements [7]. The results still require confirmation by cloning and sequencing of the whole SCCmec cassette for these six novel patterns.

Table 3. The composition of SCCmec elements of six SCCmec patterns in methicillin-resistant Staphylococcus epidermidis

<table>
<thead>
<tr>
<th>SCCmec elements</th>
<th>mec class</th>
<th>Amplicon size</th>
<th>Controls</th>
<th>Controls</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(kb)</td>
<td>P1 (n = 29)</td>
<td>P2 (n = 12)</td>
<td>P3 (n = 1)</td>
</tr>
<tr>
<td>ccrA1</td>
<td></td>
<td>0.7</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>ccrA2</td>
<td></td>
<td>1</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>ccrA3</td>
<td></td>
<td>1.6</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>ccrC</td>
<td></td>
<td>0.5</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>ccrA2-ccrA3</td>
<td></td>
<td>6.5</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>ccrA3-MTn554</td>
<td></td>
<td>9.0</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>mccf</td>
<td></td>
<td>0.180</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>mecR1 (PB)</td>
<td>A</td>
<td>0.320</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>mecR1 (MS)</td>
<td>A and B</td>
<td>0.310</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>IS272-mecA</td>
<td>B</td>
<td>2</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>dse region-mecA</td>
<td></td>
<td>4.6</td>
<td>+</td>
<td>–</td>
<td>ND</td>
</tr>
<tr>
<td>mecI-mecA</td>
<td></td>
<td>2.280</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>mecR (PB)-mecA</td>
<td>A</td>
<td>3.9</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>IS272-mecR1 (MS)</td>
<td>B</td>
<td>1.7</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>IS272-mecI</td>
<td></td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>mecR (PB)-mecR1</td>
<td></td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>mec operon</td>
<td></td>
<td>1.5</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>TnpA</td>
<td></td>
<td>0.4</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

+, amplified; –, not amplified; ND, not determined.

Table 3 footnote:

- Estimated size of the amplicon.
- SCCmec patterns are based on the finding of eight loci (A–H).
- SCCmec types of methicillin-resistant Staphylococcus aureus: III, IIIA, IIIB and IV.

Fig. 3. Pulsed-field gel electrophoresis (PFGE) dendrogram of the Finnish methicillin-resistant Staphylococcus epidermidis strains. The scale bar at the top of the dendrogram indicates similarity.
The molecular genotypes of 60 representative MRSE isolates from two distinct time periods (1990–1993 and 1997–1998) and two hospitals (Table 2) were also studied. A limited heterogeneity in the genomes was discovered: (i) there were 11 PFGE types among 60 isolates; and (ii) all PFGE types fell into one MLST sequence type, ST2. These findings suggest that a single clone has dominated in the two hospitals for nearly a decade, which has resulted in PFGE type variability over the years. MRSE with ST2 is known to have circulated in various other countries [1,14,26]. However, following analysis of the same strains using the old MLST scheme of Wisplinghoff [13], three different STs were found. Ten strains had identical STs, and two strains (FIN-SE-4 and FIN-SE-7) had unique STs (data not show). The old scheme differs from the new by five of the seven target alleles. This finding might suggest that the old MLST scheme [13] is more discriminatory than the new one [26], at least with respect to the material studied here.

Besides five unique PFGE profiles (FIN-SE-2, FIN-SE-3, FIN-SE-7, FIN-SE-8 and FIN-SE-9) found in the NICU and two (FIN-SE-6 and FIN-SE-10) in the PHU, one PFGE profile (FIN-SE-1) was shared between isolates in the NICU and PHU. The AHU consists of two wards (A and B) located on different floors with separate nursing staffs. Doctors, however, are shared. Movement of patients between the two wards is rare. The current results indicate that both AHU wards had isolates with unique PFGE profiles; FIN-SE-5, FIN-SE-6 and FIN-SE-11 in AHU-A, and FIN-SE-1 and FIN-SE-4 in AHU-B (Table 2).

This study, which is one of the first to describe the composition of SCCmec in S. epidermidis, highlights the diversity of SCCmec types in MRSE. We suggest that the MRSE strains carry elements of two cassette types: SCCmec type IV (or IVA) and SCCmec IIIA (or IIIB). These are similar to the SCCmec types of MRSA. This study is in agreement with other previous studies [1,10,14,27,30,33] that have reported the high variability of SCCmec types in S. epidermidis.

TRANSPARENCY DECLARATION

This work was supported by grants from the Paulo Foundation and the Maud Kuistila Memorial Foundation. All authors declare they do not have any commercial or other association that might pose a conflict of interest regarding this manuscript.

REFERENCES

25. Enright MC, Day NP, Davies CE, Peacock SJ, Archer GL, Enright MC. Multilocus sequence typing for characteriza-