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Carriage of Methicillin-Resistant Staphylococci and Their SCC*mec* Types in a Long-Term-Care Facility▽

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Following an outbreak caused by staphylococcal cassette chromosome *mec* (SCC*mec*) 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 SCC*mec* 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 SCC*mec* 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 SCC*mec* 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 SCC*mec* type V among their isolates. Nasal MR-CNS carriage was common among the residents of this long-term-care facility. A variety of SCC*mec* types, including many new types, were identified among the MR-CNS strains. The horizontal transfer of SCC*mec* elements is speculated based on the sharing of SCC*mec* 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 (PBP2′), which is encoded by the *mecA* gene. In *S. aureus* and CNS, *mecA* is located on a genetic element called the staphylococcal cassette chromosome (SCC*mec*) (15, 37). SCC*mec* is integrated into the chromosome of *S. aureus* at a unique site (*attBscc*) located near the *S. aureus* origin of replication. Up to now, six different SCC*mec* 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 SCC*mec* 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 SCC*mec* 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 SCC*mec* 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 SCC*mec* type V (17). In this study, we focused on the structure of the SCC*mec* elements of methicillin-resistant (MR)-CNS strains and a structural comparison of SCC*mec* elements of methicillin-resistant *S. epidermidis* (MRSE) and MRSA isolated from the same person at the same time, under the suspicion of horizontal SCC*mec* 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

Strain	No. of MRSA isolates	No. of MSSA isolates	No. of CNS isolates	Total (n = 73)
MR-CNS	2	11	28	41
MS-CNS[a]	1	4	21	26
S. aureus	2	4		6

[a] MS, methicillin susceptible.

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MATERIALS AND METHODS

Setting. A 34-bed health care ward, situated in a municipality of 5,000 inhabitants in northern Finland, takes care of the elderly patients with multiple underlying diseases, but it also gives primary care. The associated 46-bed nursing home is only for the elderly. Each room has four patients. A total of 76 nasal swabs were collected from 76 out of the total of 80 patients on 26 February 2004. One swab per patient was taken from both nostrils. The median age of 76 patients was 80 years (range, 35 to 99 years), 36% were male (n = 27), 26% used antimicrobials (n = 20), and 5% used foreign devices (n = 4). The median length of nursing stay was 9 months (range, <1 to 90 months).

Bacterial cultures and identification of staphylococci. The screening swabs (Probact transport swab; Schofield St-Heywood, United Kingdom) were cultivated on nonselective sheep blood agar (SBA; CM1008; Oxoid, United Kingdom) and on selective oxacillin resistance screening agar (ORSAB; CM1008; Oxoid, United Kingdom) plates. The SBA plates were incubated for 48 h and ORSAB plates were incubated for 96 h, and they were inspected daily. Based on colony morphology, staphylococcus-like colonies were picked and subcultured onto the SBA plate. The colonies were identified by conventional biochemical tests (1, 18). If the identification of staphylococcal species by using these tests was unclear, GenoType *Staphylococcus* (Hain Lifescience, Germany) was performed. For all CNS isolates, resistance to methicillin was determined by the oxacillin disk diffusion test (inhibition zone, ≤18 mm), and the oxacillin MIC (Etest; AB Biodisk, Solna, Sweden) was tested for every MR-CNS isolate (4).

SCC*mec* typing. The SCC*mec* types were determined by two PCR methods. The first multiplex PCR method, modified slightly (11) from the original description (31) by Oliveira and de Lencastre, detects eight loci (A through H) within SCC*mec* and uses *mecA* as an internal control. Based on the first PCR, representative isolates (in each MR-CNS species) of the different SCC*mec* patterns, and four isolates from which only *mecA* was amplified, were analyzed for their *ccr* and *mec* components by using the multiplex PCR methods described by Kondo and coworkers (19). This assay identifies *mecA* and the *ccr* types (*ccrAB1* to *ccrAB4* and *ccrC*) as well as the *mec* classes A, B, and C. The following reference strains were used in the analysis: Iberian HPV107 (SCC*mec* type IA, *ccrA1*, class B), UK EMRSA-16 96/32010 (SCC*mec* type II, *ccrA2*, class A), Brazilian HSJ216 (SCC*mec* type IIIA, *ccrA3*, class A), Pediatric clone HDE288 (SCC*mec* type VI, *ccrA4*, class B) (32), and the Finnish MRSA FIN-7 (SCC*mec* type V, *ccrC*, class C) (18).

PFGE. Pulsed-field gel electrophoresis (PFGE) was carried out as previously described for *S. aureus* (29). PFGE patterns were analyzed by BioNumerics (version 2.0; Applied Maths, Kortrijk, Belgium) and were further interpreted according to the criteria of Tenover et al. (35).

Ethical aspects. We were at liberty to collect the samples from the residents with approval from the Ministry of Social Affairs and Health and the data protection authority. In addition, permission for sampling was asked from each patient.

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

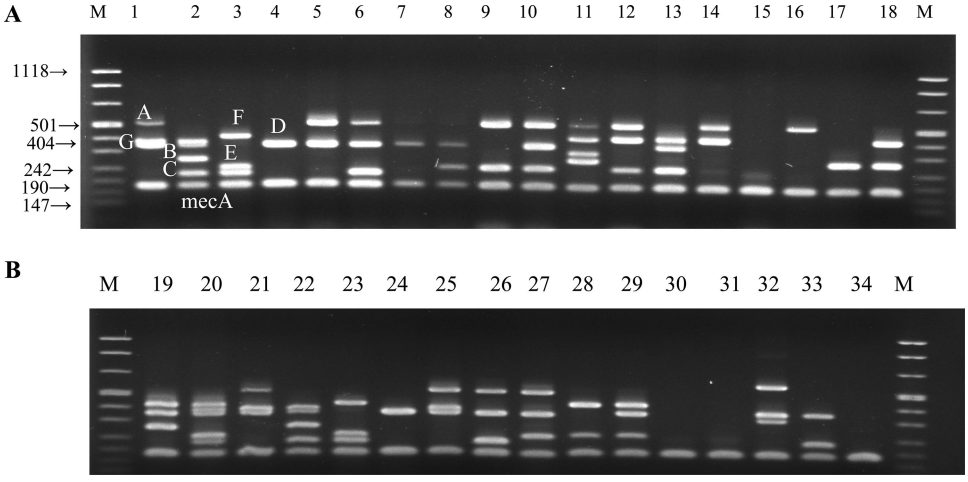

FIG. 1. SCC*mec* multiplex patterns of MR-CNS strains (31). M, molecular size markers (in kilodaltons). Lanes 1 to 4 and 21 to 24, control MRSA of type IA (locus A, upstream of the *pls* gene; locus G, left of the junction between IS*431* and pUB*110*; and locus D, the *dcs* region), type II (loci G, D, and B, *kdp* operon; locus C, *mecI* gene), type IIIA (locus F, between Tn*554* and *orfX*; locus E, between integrated pI258 and Tn*554*; and locus C) and SCC*mec* type IV (locus D) and internal control *mecA*. The sizes of amplicons, by locus, are the following: A, 495 bp; B, 284 bp; C, 209 bp; D, 342 bp; E, 243 bp; F, 414 bp; G, 381 bp; and *mecA*, 160 bp. Lanes 5 to 20, MRSE isolates; lanes 25 to 31 and 33, methicillin-resistant *S. capitis*; lane 32, methicillin-resistant *S. haemolyticus*; and lane 34, methicillin-resistant *S. hominis*.

TABLE 2. Distribution of the SCC*mec* types or elements within the CNS isolates

Staphylococcal isolate(s)	SCC*mec* type	Locus (loci) amplified by the Oliveira strategy (31)	Locus (loci) amplified by the Kondo strategy (19)		No. of isolates
			ccr type	*mec* class	
S. epidermidis (n = 49)	I	*mecA*, D, A	1	B	3
	II	*mecA*, C, D, A	2	A	2
	IV	*mecA*, D	2	B	2
	IV	*mecA*, E, D	2	B	2
	IV	*mecA*, E, A	2	B	1
	IV	*mecA*, E, D, A	2	B	13
	V	*mecA*, B, H, F, A	*ccrC*	C	1
	V	*mecA*, E, F, A	*ccrC*	C	3
	V	*mecA*, E (very faint), F, A	*ccrC*	C	5
	New (IV + *ccrC*)	*mecA*, E, D, F	2, *ccrC*	B	1
	New (V + *ccrA4*)	*mecA*, F, A	4, *ccrC*	C	1
	NT[a]	*mecA*	None	None	1
	NT	*mecA*, A	None	None	1
	NT	*mecA*, B	None	None	1
	New	*mecA*, B, F	*ccrC*	A	8
	New	*mecA*, B, D, F	4, *ccrC*	A	3
	New	*mecA*, C, E, D, G, F	3, 4, *ccrC*	A and B	1
S. capitis (n = 10)	IA	*mecA*, D, G, A	1	B	1
	II	*mecA*, C, D, A	2	A	1
	IV	*mecA*, E, D, A	2	B	1
	V	*mecA*, E, F	*ccrC*	C	2
	New (IV + *ccrC*)	*mecA*, E, D, F	2, *ccrC*	B	2
	New	*mecA*	1	A	2
	New	*mecA*, C, D	3, 4	A and B	1
S. haemolyticus (n = 1)	IV	*mecA*, D, H, A (>501 bp)	2	B	1
S. hominis (n = 1)	New	*mecA*	1	A	1

[a] NT, nontypeable.

classified as SCC*mec* type I (Fig. 1, lane 5) and two as SCC*mec* 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 SCC*mec* types (31). Within the other MR-CNS species, one methicillin-resistant *S. capitis* isolate could be classified as SCC*mec* 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 any previously known SCC*mec* 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 SCC*mec* type I, 2 (4%) type II, 18 (37%) type IV, and 9 (12%) type V. Three isolates were of a nontypeable SCC*mec* 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 SCC*mec* types IA, II, and IV, and two harbored type V. The remaining five isolates harbored a new SCC*mec* type (Table 2). The single methicillin-resistant *S. haemolyticus* isolate harbored SCC*mec* type IV, and the methicillin-resistant *S. hominis* isolate harbored a new SCC*mec* 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 SCC*mec* type V (*ccrC* and class C), while the two MRSE strains were of different SCC*mec* types, type II (*ccrA2* and class A) and a new SCC*mec* type (*ccrC* and *mec*, class A). The second carrier had MRSA and MRSE, both of which harbored SCC*mec* type V (Fig. 2B and C and 3). The MRSE multiplex SCC*mec* 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 SCC*mec* types, with many new combinations of elements as well as nontypeable types, were recognized among the MR-CNS strains. The horizontal transfer of SCC*mec* elements is speculated based on the sharing of SCC*mec* 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 SCC*mec* typing of MR-CNS isolates revealed that 62%

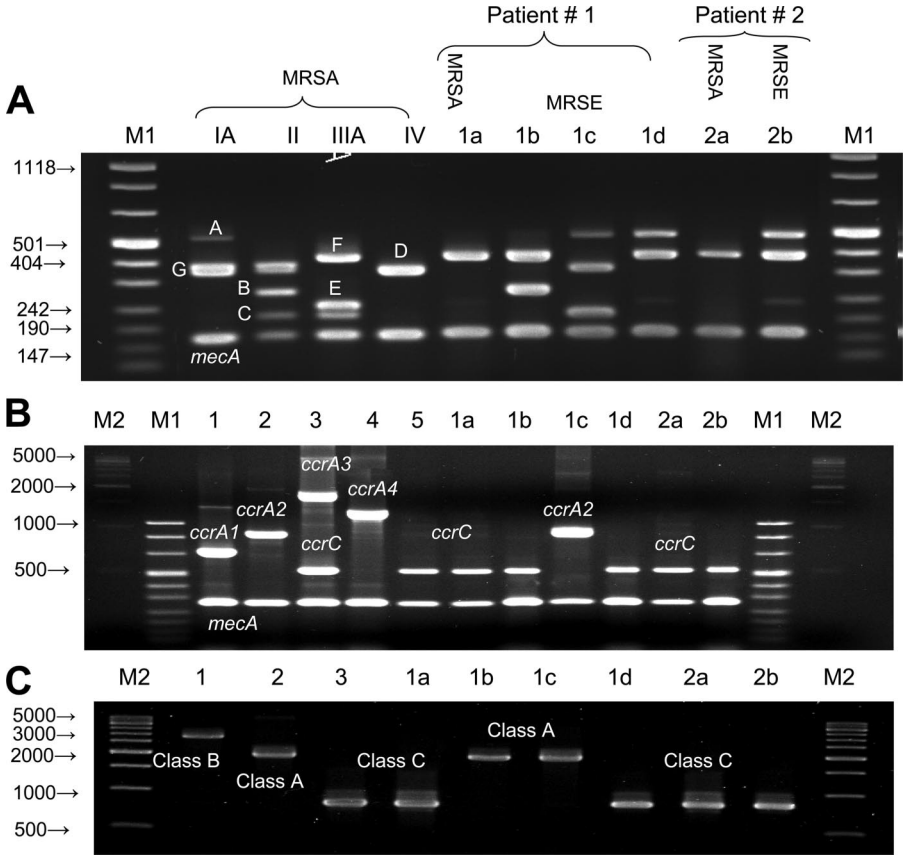

FIG. 2. Three multiplex PCRs for the SCC*mec* type assignment of the staphylococcal isolates from the two patients colonized with MRSA and MRSE. Lanes M1 and M2, molecular size markers. Lanes 1 to 5, control MRSA for SCC*mec* type IA (*ccrA1*, class B), SCC*mec* type II (*ccrA2*, class A), SCC*mec* type IIIA (*ccrA3*, class A), SCC*mec* type VI (*ccrA4*, class B), and SCC*mec* type V (*ccrC* and class C). (A) Multiplex PCR patterns (31). Lanes 1 to 4, controls; lanes 1a and 2a, MRSA isolates; lanes 1b, 1c, 1d, and 2b, MRSE isolates. (B) Multiplex PCR for the typing of *ccr* genes. Lanes 1 to 5, control MRSA isolates for *ccr* types. (C) Multiplex PCR for typing of *mec* genes (19). Lanes 1 to 3, control MRSA isolates for *mec* classes. Lanes 1a and 2a, MRSA (*ccrC*, class C); lane 1b, MRSE (*ccrC*, class A); lane 1c, MRSE (*ccrA2*, class A); lanes 1d and 2b, MRSE (*ccrC*, class C); lane 2a, MRSA (*ccrC*) and MRSE (*ccrC*).

of the isolates harbored previously recognized SCC*mec* 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 SCC*mec* type IV, and SCC*mec* type IV was most prevalent among the *S. epidermidis* strains (37%). Among the 20 strains, 18 harbored a modified SCC*mec* 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 SCC*mec* types; SCC*mec* type V was found among 11 MR-CNS strains, which represented four different multiplex SCC*mec* patterns (Fig. 1 and

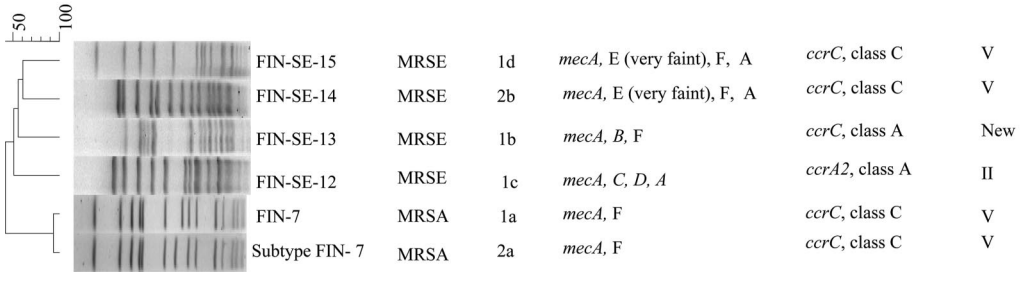

FIG. 3. PFGE dendrogram of the methicillin-resistant staphylococcal isolates from two patients colonized with MRSA and MRSE. The distribution of the SCC*mec* types/elements for each strain is included. The scale bar at the top of the dendrogram represents similarity.

Table 2). Three MR-CNS strains harboring SCC*mec* type II did not have locus B (*kdp* operon). The remaining 23 strains harbored a new SCC*mec* type; these strains carried either (i) known SCC*mec* types with additional elements (i.e., type IV and *ccrC* or type V and *ccrA4*) or (ii) combinations of *ccr* and *mec* that could not be interpreted as belonging to any of the presently described SCC*mec* types (Table 2). Previous studies also have shown variations in SCC*mec* cassettes. These include (i) strains containing both SCC*mec* 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 SCC*mec* 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 SCC*mec* type. For instance, locus A was previously thought to be part of SCC*mec* type I and IA only, but according to this study, it also is present in types II, IV, and V. Moreover, the SCC*mec* types IV and V contained a variety of loci. Locus B previously has been defined to be specific for SCC*mec* 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 SCC*mec* 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 SCC*mec* 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 SCC*mec* 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 SCC*mec* cassettes were acquired through different routes, this observation supports the possibility of SCC*mec* transfer. If such a transfer has happened, it was not complete. Further studies revealing the mechanisms of SCC*mec* transfer are needed. The hypothesis for the transfer of SCC*mec* between *S. epidermidis* and *S. aureus* has been previously reported (6, 7, 27, 37).

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