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Distribution of Staphylococcal Cassette Chromosome (SCC) *mec* Element Types in Fusidic Acid-Resistant *Staphylococcus epidermidis* and Identification of a Novel SCC_{7684} Element

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We analyzed the staphylococcal cassette chromosome *mec* (SCC*mec*) types of 143 fusidic acid- and methicillin-resistant *Staphylococcus epidermidis* isolates. The most frequent SCC*mec* type was SCC*mec* III/SCC*Hg* (53%), followed by SCC*mec* IV (29%). Clonal spreading of SCC*mec* III/SCC*Hg* strains contributed to the increased prevalence of SCC*mec* III. A novel non-*mec* SCC structure, SCC_{7684}, adjacent to SCC*mec* III, which carries a new *ccrC* allotype (*ccrC3* allele 1) and contains heavy metal resistance genes, was identified in 14 isolates.

Methicillin resistance in staphylococci results from the production of an alternative penicillin-binding protein 2a (PBP 2a) with low affinity for β-lactam antibiotics, encoded by the *mecA* gene. The *mecA* gene is located on a mobile genetic element known as the staphylococcal cassette chromosome *mec* (SCC*mec*) (1). It has been suggested that methicillin-susceptible *Staphylococcus aureus* acquires SCC*mec* from methicillin-resistant coagulase-negative staphylococci (CoNS) and becomes methicillin-resistant *S. aureus* (MRSA) (2, 3). The structures and types of SCC*mec* in CoNS are usually more complex than in *S. aureus*. In addition to SCC*mec*, non-*mec* SCCs composed of *ccr* genes and resistance genes other than *mecA* have been reported, such as SCC*Hg*, SCC*fusC* (4), and SCC*pbp4* (5).

We previously found that the majority of fusidic acid-resistant *Staphylococcus epidermidis* isolates were also resistant to methicillin (6). To understand the distribution of SCC*mec* types, a total of 155 fusidic acid-resistant *S. epidermidis* isolates, including 141 clinical isolates that were collected between 2008 and 2010 in the Bacteriology Laboratory of the National Taiwan University Hospital (6) and 14 commensal isolates (7), were tested. The *mecA* gene was detected in 143 isolates, including 137 clinical isolates and 6 commensal isolates. Among 137 clinical isolates, 132 carried *fusB*, 4 carried *fusC*, and 1 had an *fusA* point mutation, all of which have been studied and published (6). All of the 6 commensal methicillin-resistant isolates carried *fusB*.

The SCC*mec* types of 143 *mecA*-positive isolates were determined by standard methods (1, 8–12), and the results are listed in Table 1. The most frequent SCC*mec* type was SCC*mec* III/SCC*Hg* ($n = 76$, 53%), followed by SCC*mec* IV ($n = 41$, 29%). This result is different from those of other reports in which SCC*mec* IV was usually dominant in methicillin-resistant *S. epidermidis* (MRSE) (13, 14). However, the isolates tested in the present study were all fusidic acid-resistant rather than general MRSE. There were 17 SCC*mec* III isolates that lacked SCC*Hg*. Of them, 3 carried only SCC*mec* III, and 14 contained an additional novel structure, SCC_{7684} (described later). All of the 6 *mecA*-positive commensal isolates were SCC*mec* type IV. An additional *ccr* gene was found in 2 SCC*mec* type IV/*ccrA1B1*, 1 SCC*mec* type IV/*ccrC*, and 2 SCC*mec* type IV/SCC*fusC* isolates. One SCC*mec* type V_T/SCC*fusC* was identified. It is not uncommon for *mecA*-positive CoNS to carry multiple *ccr* copies or no *ccr* genes (3, 15, 16). SCC*fusC* adjacent to SCC*mec* III was previously found in MRSA (4). In the present study, SCC*fus*C was linked to SCC*mec* IV or SCC*mec* V_T in *S. epidermidis*, which has not been reported before, suggesting that insertions of SCC*mec* and SCC*fusC* were independent. In 4 SCC*mec* nontypeable isolates, no *ccr* gene was detected. For SCC*mec* nontypeable isolates, there are two possibilities: the isolates may have a novel type or the target sites for the primers may have been altered. In a study in Norway, *ccr*-nontypeable *S. epidermidis* isolates were reported 52% of the time (3).

Pulsed-field gel electrophoresis (PFGE) analysis divided 155 *S. epidermidis* isolates (143 MRSE and 12 methicillin-susceptible *S. epidermidis* [MSSE] isolates) into 43 clusters (Table 1). Pulsotype D was the most frequent (37/155, 24%), followed by pulsotype A (25/155, 16%). Most of the isolates that carried SCC*mec* III/SCC*Hg* belonged to pulsotypes A (21/76, 28%) and D (36/76, 47%). Clonal spreading of SCC*mec* III/SCC*Hg* strains may contribute to the increased prevalence of SCC*mec* III. Eleven of 14 isolates that contained SCC*mec* III/SCC_{7684} were pulsotype I. Isolates of SCC*mec* IV were distributed among different pulsotypes. Six commensal isolates that carried SCC*mec* IV were distributed in the following three pulsotypes: T, AB, and AQ. Some isolates with different SCC*mec* types clustered in the same pulsotype, such as pulsotype A (SCC*mec* III/SCC*Hg*, 21 isolates; SCC*mec* III, 2 isolates; SCC*mec* IV, 1 isolate; SCC*mec* VIII, 1 isolate), indicating the possibility of the intraspecies transfer of SCC*mec*.

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TABLE 1 SCC*mec* types and pulsotypes among 143 fusidic acid-resistant *S. epidermidis* isolates

SCC*mec* type	Other SCC, additional *ccr* gene, or *mec* complex	No. of isolates	PFGE pattern(s) (no. of isolates)
III	SCC*Hg*	76	A (21), B (6), D (36), E (1), F (8), G (2), AC (1), AM (1)
III		3	A (2), D (1)
III	SCC_{7684}	14	I (11), J (1), N (1), P (1)
IV		36	A (1), H (1), K (1), Q (3), T (2),[b] V (2), W (1), X (1),Y (2), Z (2), AA (4), AB (1),[b] AD (2), AE (2), AF (3), AG (3), AH (1), AI (1), AQ (3)[b]
	ccrA1B1	2	Q (1), R (1)
	ccrC	1	L (1)
	SCC*fusC*	2	X (1), Y (1)
IV variant[a]		3	L (1), M (1), AA (1)
V_T	SCC*fusC*	1	X (1)
VIII		1	A (1)
NT1	*mec* complex A	3	I (1), N (1), U (1)
NT2	*mec* complex B	1	O (1)
Total		143	

[a] This type is the large size of *mec* complex B.
[b] These are commensal isolates collected from healthy volunteers.

A total of 17 isolates were SCC*mec* type III but lacked SCC*Hg*, which was the third most frequent type after SCC*mec* III/SCC*Hg* and SCC*mec* IV. One isolate, NTUH-7684, was chosen for whole-genome sequencing to determine the possible novel composite SCC*mec* III-related element. A 56,238-bp element was found abutting the SCC*mec* III in NTUH-7684 (Fig. 1). The sequence of SCC*mec* III in NTUH-7684 showed 98.94% identity with that in *S. aureus* 85/2082, except for the truncated J3 region and the intact *mecR1* and *mecI*, which were truncated in *S. aureus* 85/2082. A novel allotype of *ccrC* was identified. The 1,686-bp *ccrC* sequence was closest to that of the *ccrC1* allele 10 in *S. aureus* TW20 (GenBank accession no. GQ902038; 69.69% identity) (Fig. 2) and showed 69.45% identity to *ccrC1* allele 2 in *S. aureus* TSGH17 (GenBank accession no. AY894416). The *ccrC* gene in NTUH-7684 is designated *ccrC3* allele 1 according to the 85% cutoff value. The phylogenetic tree based on concatenated sequences of *ccrC* genes indicated that *ccrC3* allele 1 was phylogenetically separated from *ccrC1* and *ccrC2* (Fig. 2). The *ccrC3* allele 1 was detected in 14 of 17 isolates with SCC*mec* type III lacking SCC*Hg* by PCR using a pair of primers (SE-ccrC3-359F, 5′-GCG AAATGATGATAGGAGCA-3′, and SE-ccrC3-1368R, 5′-ATTCA TAGCCTCAGCCTGC-3′). Isolates (11/14, 79%) that carried SCC*mec* III/SCC_{7684} mostly belonged to pulsotype I, indicating clonal spreading. The presence of the *ccrC3* allele 1 indicated that the element was a non-*mec* SCC, which was referred to as SCC_{7684}.

SCC_{7684} contained many heavy metal (cadmium, mercury,

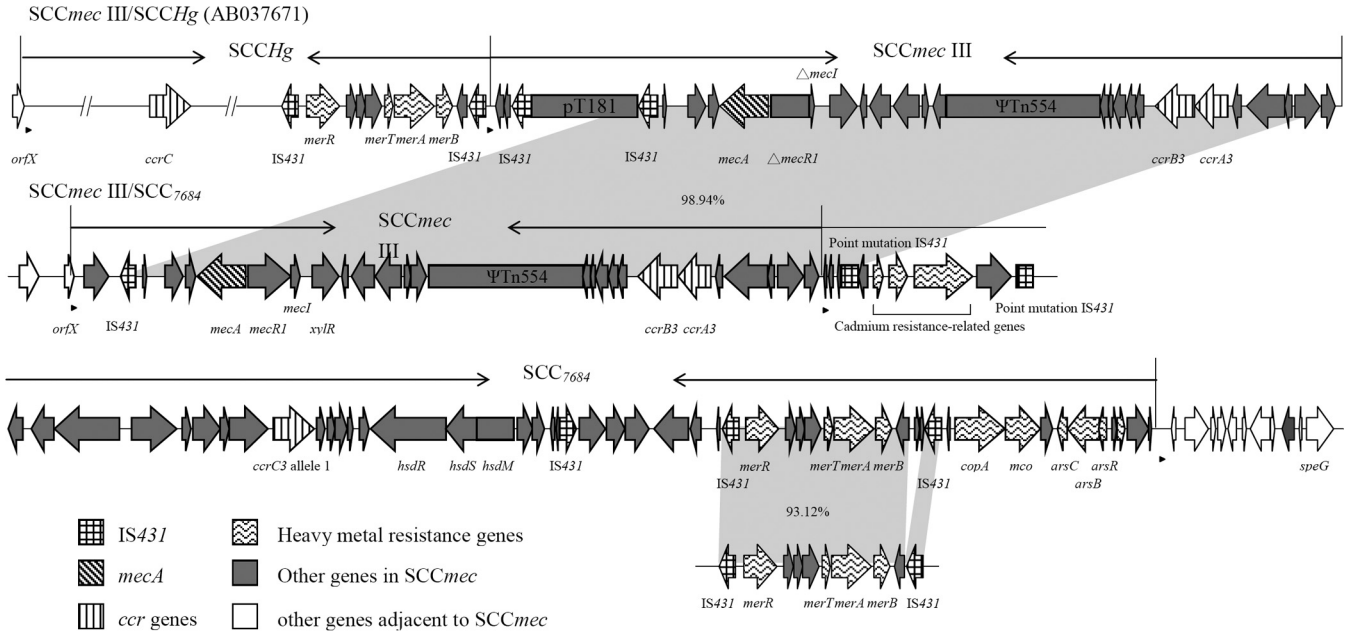

FIG 1 Genetic organization of SCC*mec* III/SCC_{7684} in *S. epidermidis* NTUH-7684 (GenBank accession no. LC085180) compared with SCC*mec* III/SCC*Hg* (GenBank accession no. AB037671) in *S. aureus*. Genes are shown according to their sequences. The predicted integration site sequences are indicated by arrows. Homologous regions between SCC*mec* are shown in shaded areas, and the numbers in the shaded areas show percent identities between the corresponding sequences.

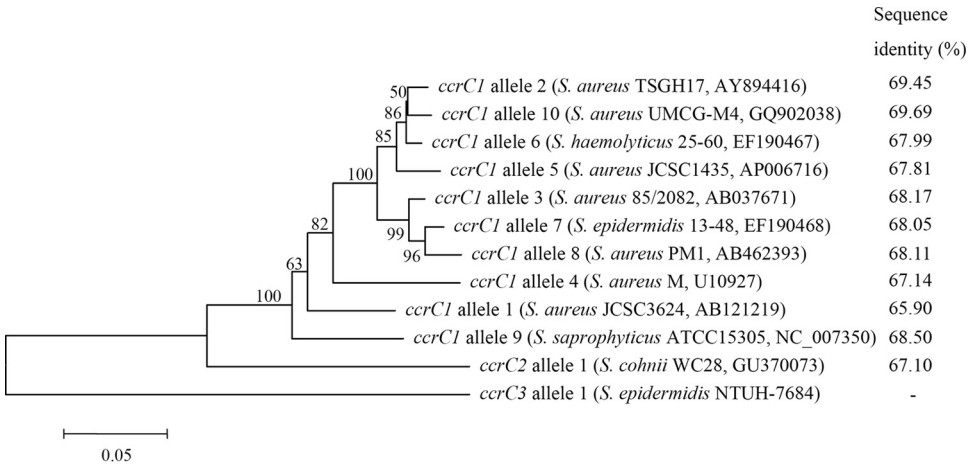

FIG 2 Phylogenetic relationships for the *ccrC* genes and sequence identity compared with *ccrC3* allele 1 in *S. epidermidis* NTUH-7684. The phylogenetic tree was generated by using the neighbor-joining method with the MEGA6 package. Numbers at nodes are confidence levels expressed as percentages of occurrence in 500 bootstrapped resamplings. Scale bars indicate the evolutionary distance between sequences, determined by measuring the lengths of the horizontal lines connecting two organisms.

copper, and arsenic) resistance genes, genes associated with type I restriction modification systems (*hsdR*, *hsdS*, *hsdM*, IS*431*), and genes encoding hypothetical proteins. The region containing mercury resistance genes flanked by IS*431* showed high similarity (93.12% identity) to the SCC*Hg* region in *S. aureus* 85/2082. The 18-bp integration site sequences (ISSs), GAAGC(A/T/G)TA(T/C)CA(T/C)AA(A/G)T(A/G)A, were found at the end of SCC*mec* III and SCC_{7684} (Fig. 1). The SCC*mec* III and SCC_{7684} elements were flanked by 15-bp imperfectly matched direct repeats (DRs), (A/C)GAAGC(A/T/G)TA(T/C)CA(T/C)AA, which have also been found in other SCC elements (17, 18), suggesting that the two SCC elements integrated into the chromosome independently.

In conclusion, this is the first study to investigate the distribution of SCC*mec* types among fusidic acid-resistant *S. epidermidis* isolates. PFGE analyses of isolates carrying SCC*mec* III/SCC*Hg* or SCC*mec* III/SCC_{7684} indicate that each showed clonal spreading. The SCC_{7684} element possessed many genes associated with heavy metal resistance, which may provide an advantage for bacterial survival. Our findings highlight the importance of characterizing the SCC-related elements in *S. epidermidis*.

Nucleotide sequence accession number. The SCC_{7684} sequence from the *S. epidermidis* clinical isolate NTUH-7684 was deposited in GenBank under accession no. LC085180.

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