

Novel Type of Staphylococcal Cassette Chromosome *mec* Identified in Community-Acquired Methicillin-Resistant *Staphylococcus aureus* Strains

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We identified a new type of staphylococcal cassette chromosome *mec* (SCC*mec*) from two community-acquired methicillin-resistant *Staphylococcus aureus* (MRSA) strains. The novel element, designated type IV SCC*mec*, had a unique combination of the class B *mec* gene complex and the type 2 *ccr* gene complex and was much smaller in size (21 to 24 kb) than previously identified SCC*mec* elements of hospital-acquired MRSA. Consistent with the strains' susceptibilities to various non- β -lactam antibiotics, the type IV SCC*mec* was devoid of any antibiotic resistance genes other than the *mecA* gene.

Since the first discovery of methicillin-resistant *Staphylococcus aureus* (MRSA) in 1961 in England, MRSA has become one of the most prevalent pathogens that cause nosocomial infections (13). MRSA produces a specific penicillin-binding protein (PBP) called PBP 2' (or PBP 2a) that possesses reduced affinities for binding to β -lactam antibiotics (2, 7, 22). PBP 2' is encoded by the *mecA* gene, which is carried by a large mobile genetic element that is designated staphylococcal cassette chromosome *mec* (SCC*mec*) and that is integrated on the chromosomes of MRSA strains isolated from hospitals in various countries throughout the world (11, 12, 14, 15).

Recently, MRSA infections have increasingly been reported among groups of patients with no apparent connection to hospitals (4). Those strains, designated community-acquired MRSA (C-MRSA) strains, have been reported in various countries such as Australia (16, 18), New Zealand (19), the United Kingdom (20), Canada (5), and the United States (6, 8). The death of four children caused by C-MRSA strains has alerted us to the threat of latent dissemination of highly virulent C-MRSA strains in the community in the United States (3).

In contrast to hospital-acquired MRSA (H-MRSA), C-MRSA is characteristically susceptible to many antibiotics (3, 21), but it remains unclear whether C-MRSA is a descendant of H-MRSA or was born and evolved independently of the hospital environment (4). In order to understand the evolutionary relationship between C-MRSA and H-MRSA, we determined the entire nucleotide sequences of the SCC*mec* elements integrated into the chromosomes of two C-MRSA clinical strains. Strain CA05 (JCSC1968) was isolated from the joint fluid of a patient with septic arthritis and osteomyelitis, and strain 8/6-3P (JCSC1978) was isolated from the perineum of another patient (10).

We amplified DNAs encompassing the entire SCC*mec* sequence by long-range PCR with several sets of primers, as follows. The region from the left extremity to the *ccr* genes (L-C region) was covered by primer sets α 5 and cLs1 (CA05) or CL2b (8/6-3P) (Fig. 1). Primers α 6 and mCr8 were used to cover the middle part (from the region just upstream of *ccrA* to *mecR1*; the C-M region). Two overlapping primer sets (primers is4 and mA2 and primers mA3 and cR1) were used to cover the right extremities (from IS431*mec* to *orfX*; the I-R region) (Fig. 1) (11, 12, 14). The rest of the element was amplified and sequenced with primers as described previously (11, 12, 14). The PCR products were purified with a High Pure PCR product purification kit (Roche Diagnostics GmbH, Mannheim, Germany), and their nucleotide sequences were determined as described previously (9).

Figure 1 illustrates the genomic organizations of the SCC*mec* elements identified from the two C-MRSA strains in comparison with the three extant types of SCC*mec*. The SCC*mec* elements of strains CA05 and 8/6-3P were 24,248 and 20,920 bp, respectively, and were much smaller (34 to 67 kb) than three types of SCC*mec* elements identified from H-MRSA strains (12). Both elements were found to be integrated at the integration site for SCC*mec*, *attB**scc*, which is found inside *orfX*, which has an unknown function and is located near the origin of replication of the *S. aureus* chromosome (12, 15). The SCC*mec* elements shared a pair of 15-bp direct repeat sequences: one (DR*scc*-R) at the right extremities and the other (DR*scc*-L) on the chromosome region abutting the left termini of the elements (shown as thick arrows in Fig. 2). Degenerate inverted repeats were also found in the extremities of the two SCC*mec* elements (shown by thin arrows in Fig. 2). The authenticity of the SCC*mec* boundaries was vindicated by using a previously described method (14); i.e., by confirming that the element was precisely cut out from the chromosomes of the two C-MRSA strains, regenerating *attB**scc* in the chromosomes of the cell populations from which it was excised.

The nucleotide sequences of the L-C regions of the two SCC*mec* elements differed from each other, but otherwise,

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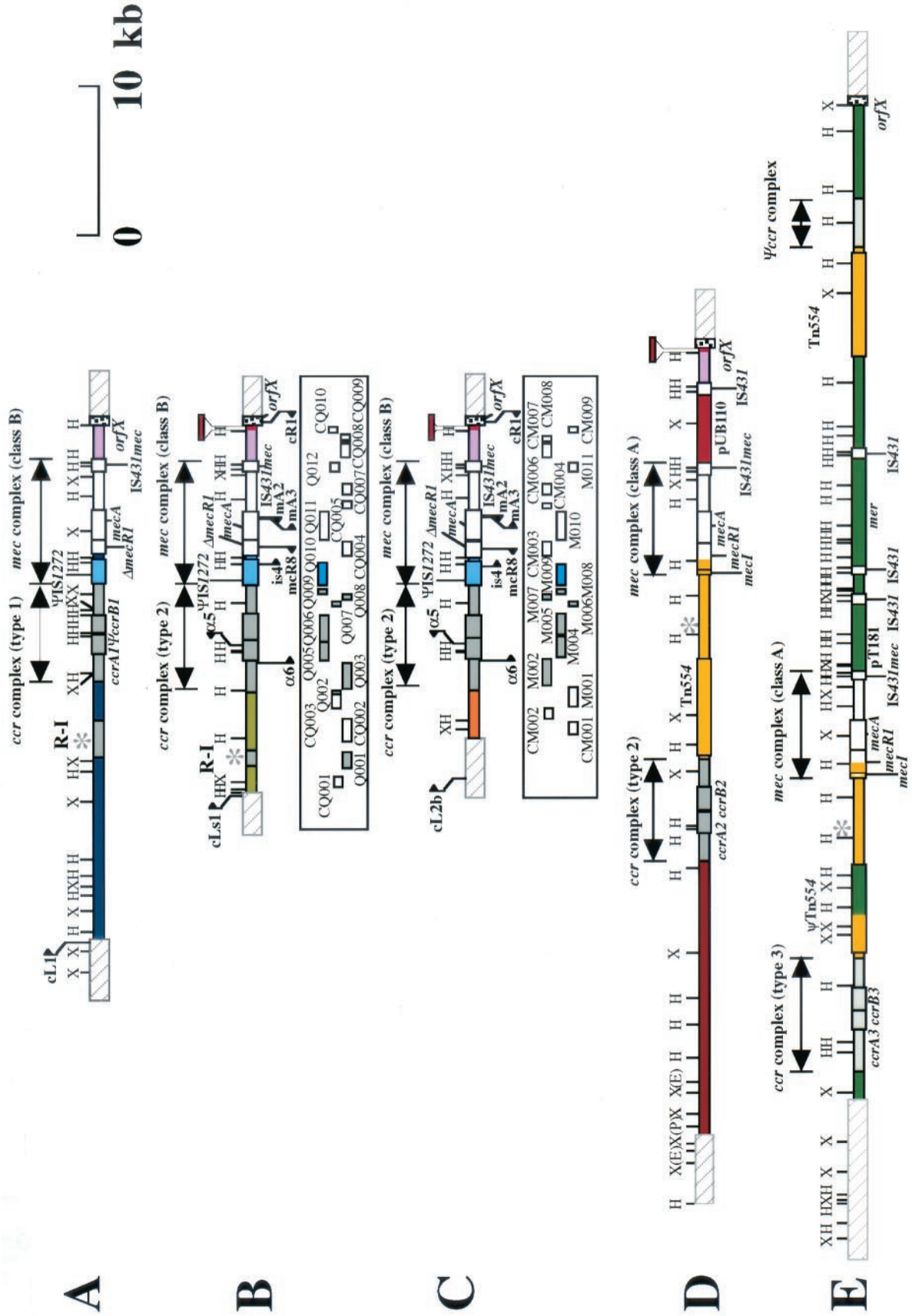


FIG. 1. Structure of the SCCmec elements identified from C-MRSA strains in comparison with the three types of SCCmec elements. (A) Type I SCCmec carried by NCTC 10442. (B) Type IV SCCmec carried by strain CA05 (subtype a). (C) Type IV SCCmec carried by strain 8/6-3P (subtype b). (D) Type II SCCmec carried by N315. (E) Type III SCCmec carried by 85/2082. The ORFs of greater than 200 nucleotides in six possible reading frames of type IV SCCmec elements are illustrated in the squares under the bars that represent essential genes and restriction sites for *Hind*III and *Xba*I. Differences in coloration correspond to differences in the nucleotide sequences. Color codes are as follows: white, ORFs or the parts of ORFs that are conserved in all four types of SCCmec elements with greater than 99% amino acid identities; gray, ORFs or the parts of ORFs that are conserved in four types of SCCmec with amino acid identities of 46 to 98%; magenta, ORFs or the parts of ORFs that are common to type I and type II SCCmec elements; yellow, ORFs or the parts of ORFs that are common to type II and type III SCCmec elements; blue, ORFs or the parts of ORFs that are unique to type I SCCmec; red, ORFs or the parts of ORFs that are unique to type II SCCmec; green, ORFs or the parts of ORFs that are unique to type III SCCmec, light green, ORFs or the parts of ORFs that are unique to type IVa SCCmec; and orange, ORFs or the parts of ORFs that are unique to type IVb SCCmec. The locations of primers used for amplification of the type IV SCCmec are indicated by arrows: they are primers $\alpha 5$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 6$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 7$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 8$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 9$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 10$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 11$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 12$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 13$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 14$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 15$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 16$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 17$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 18$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 19$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 20$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 21$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 22$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 23$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 24$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 25$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 26$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 27$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 28$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 29$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 30$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 31$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 32$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 33$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 34$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 35$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 36$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 37$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 38$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 39$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 40$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 41$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 42$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 43$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 44$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 45$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 46$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 47$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 48$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 49$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 50$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 51$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 52$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 53$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 54$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 55$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 56$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 57$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 58$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 59$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 60$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 61$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 62$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 63$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 64$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 65$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 66$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 67$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 68$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 69$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 70$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 71$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 72$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 73$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 74$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 75$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 76$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 77$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 78$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 79$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 80$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 81$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 82$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 83$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 84$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 85$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 86$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 87$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 88$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 89$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 90$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 91$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 92$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 93$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 94$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 95$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 96$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 97$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 98$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 99$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'), $\alpha 100$ (5'-TGTTAAGTATATGCACTTTATGATTCAATGCCT-3'). The entire nucleotide sequences of the type IV SCCmec elements are available in the DDBJ/EMBL/GenBank databases under accession no. AB063172 (subtype a) and AB063173 (subtype b). H, *Hind*III; X, *Xba*I; E, *Eco*RI; P, *Pst*I.

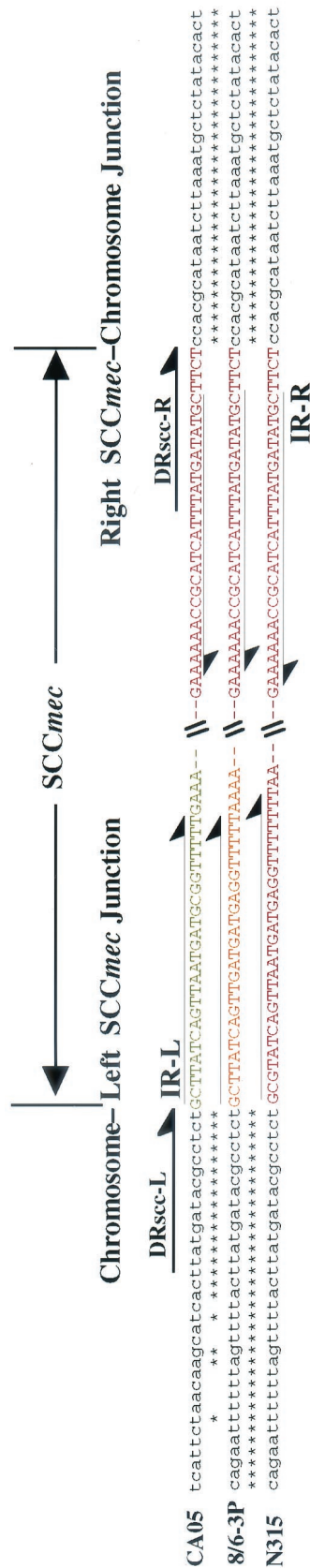


FIG. 2. Chromosome-SCCmec junction sequences of type IV SCCmec. The nucleotide sequences around the left and right boundaries of the SCCmec elements of CA05 and 8/6-3P are aligned with those of type II SCCmec. Thin arrows indicate inverted repeats IR-L and IR-R at both extremities of SCCmec elements. Thick arrows indicate direct repeats DRscc-L and DRscc-R.

TABLE 1. ORFs in SCCmec's of CA05 and 8/6-3P

Strain (element) and ORF ^a	Location (position) ^b	Size (bp) of gene	Description of gene product
CA05 (JCSC1968) (type IV SCCmec subtype a)			
CQ001	Complement (1280–1900)	621	Hypothetical protein
Q001	2653–3657	1,005	Hypothetical protein
CQ002	Complement (4281–5771)	1,491	Hypothetical protein
CQ003	Complement (6389–7438)	1,050	Conserved hypothetical protein
Q002	7577–7867	291	Conserved hypothetical protein
Q003	7867–9654	1,788	Conserved hypothetical protein
Q005	9888–11237	1,350 (<i>ccrA2.1</i>)	Cassette chromosome recombinase A
Q006	11259–12887	1,629 (<i>ccrB2.1</i>)	Cassette chromosome recombinase B
Q007	13409–13759	351	Conserved hypothetical protein
Q008	13846–14157	312	Conserved hypothetical protein
Q009	14169–14678	510	Conserved hypothetical protein
Q010	14679–16337	1,659	Putative transposase of IS 1272
CQ004	Complement (16560–17546)	987 (<i>ΔmecR1</i>)	Truncated signal transducer protein MecR1
Q011	17646–19652	2,007 (<i>mecA</i>)	PBP 2'
CQ005	Complement (19698–20126)	429	Conserved hypothetical protein
CQ007	Complement (20223–20966)	744 (<i>ugpQ</i>)	Glycerophosphoryldiester phosphodiesterase
Q012	22268–22942	675 <i>tnp</i>	Transposase for insertion sequence-like element IS431me ^c
CQ008	Complement (22974–23213)	240	Conserved hypothetical protein
CQ009	Complement (23628–24659)	1,032	Truncated conserved hypothetical protein
CQ010	Complement (24509–24883)	375	Truncated conserved hypothetical protein
(CQ011)	Complement (25205–25684)	480	OrfX
8/6-3P (JCSC1978) (type IV SCCmec subtype b)			
CM001	Complement (325–1446)	1,122	Hypothetical protein
CM002	Complement (1424–2194)	771	Hypothetical protein
M001	2311–3741	1,431	Hypothetical protein
M002	3861–5651	1,791	Conserved hypothetical protein
M004	5885–7234	1,350 (<i>ccrA2.2</i>)	Cassette chromosome recombinase A
M005	7256–8884	1,629 (<i>ccrB2.2</i>)	Cassette chromosome recombinase B
M006	9406–9756	351	Conserved hypothetical protein
M007	9843–10154	312	Conserved hypothetical protein
M008	10166–10675	510	Conserved hypothetical protein
M009	10676–12334	1,659	Putative transposase of IS1272
CM003	Complement (12557–13543)	987 (<i>ΔmecR1</i>)	Truncated signal transducer protein MecR1
M010	13643–15649	2,007 (<i>mecA</i>)	PBP 2'
CM004	Complement (15695–16123)	429	Conserved hypothetical protein
CM006	Complement (16220–16963)	744 (<i>ugpQ</i>)	Glycerophosphoryldiester phosphodiesterase
M011	18265–18939	675 <i>tnp</i>	Transposase for insertion sequence-like element IS431me ^c
CM007	Complement (18971–19210)	240	Conserved hypothetical protein
CM008	Complement (19625–20656)	1,032	Truncated conserved hypothetical protein
CM009	Complement (20506–20880)	375	Truncated conserved hypothetical protein
CM010	Complement (21204–21683)	480	OrfX

^a ORFs shown in parentheses were located outside of SCC *mec*.

^b The nucleotide position in the nucleotide sequences deposited under DDBJ/EMBL/GenBank accession no. AB063172 (type IV subtype a) and AB063173 (type IV subtype b).

^c Identity to amino acid sequences of each ORF.

^d Incomplete ORFs that are potentially defective genes or pseudogenes containing either a deletion, a nonsense mutation, or a frameshift mutation.

they shared the same features: type 2 *ccr* gene complexes, the class B *mec* gene complexes (IS1272-*ΔmecR1-mecA-IS431*), and the I-R region that was 99.9% identical to that of type II SCC*mec* elements (Fig. 1). This combination of *ccr* and *mec* gene complexes was a novel one. That is, the class B *mec* gene complex has been found to be in close linkage with the type 1 *ccr* gene complex in type I SCC*mec* (12). However, the *ccr* gene complexes of the novel SCC*mec* elements were more similar to the type 2 gene complex than to the type 1 *ccr* gene complex: the amino acid identities between the novel CcrA proteins and the type 2 CcrA (CcrA2) and type 1 CcrA (CcrA1) proteins

were about 98 and 75%, respectively; and those between the novel CcrB proteins and the CcrB2 and CcrB1 proteins were 97 to 99 and 72 to 81%, respectively (Table 1). Accordingly, we classified the genes as variants of *ccrA2* and *ccrB2* by designating them the *ccrA2.1* and *ccrA2.2* genes and the *ccrB2.1* and *ccrB2.2* genes, respectively (Table 1). As shown in Table 1, the predicted proteins encoded by the open reading frames (ORFs) surrounding the *ccrA* and *ccrB* genes also had significantly higher degrees of similarity to those of the type 2 gene complex than to those of the type 1 *ccr* gene complex. On the basis of this unique combination of the two complexes, we

TABLE 1—Continued

Homology to ORFs of type I SCCmec		Homology to ORFs of type II SCCmec		Homology to ORFs of type III SCCmec		Homology to ORFs of type IV SCCmec subtype b	
% Identity ^c	Corresponding ORF(s) (size [bp])	% Identity ^c	Corresponding ORF(s) (size [bp])	% Identity ^c	Corresponding ORF(s) (size [bp])	% Identity ^c	Corresponding ORF(s) (size [bp])
96.4	E024 (1329)	72.4	N053 ^d (861)	72.4	Z025 ^d (861)		
46.2	E025 (297)	46	N030 (297)	77.8	CZ002 (1,068)		
77.3	E026 (1,770)	85.3	N031 (1,794)	45.7	Z003 (288)		
75	<i>ccrA1</i> (1,350)	97.6	<i>ccrA2</i> (1,350)	66.3	Z004 (1,569)	82.4	M002 (1791)
72.4	* <i>ccrB1</i> (1,152)	97.4	<i>ccrB2</i> (1,629)	72.4	<i>ccrA3</i> (1,350)	98.2	M004 (1350)
85.3	E031 (351)	88.8	N041 (351)	87.5	<i>ccrB3</i> (1,629)	99.3	M005 (1629)
85.4	E032 (327)	89.2	N042 (312)	51.3	Z011 (351)	100	M006 (351)
97.6	E033 (510)	92.7	N043 (318)	47.2	Z013 (396)	100	M007 (312)
100	E034 (1,659)			61.3	Z014 (522)	100	M008 (510)
100	Δ <i>mecRI</i> _{NCTC10442} (987)	100	<i>mecRI</i> _{N315} (1,758)	100	Δ <i>mecRI</i> _{85/2082} (114)	100	M009 (1659)
99.9	<i>mecA</i> _{NCTC10442} (2,007)	100	<i>mecA</i> _{N315} (2,007)	99.7	<i>mecA</i> _{85/2082} (2,007)	100	
99.3	CE025 (429)	100	CN038 (429)	100	CZ029 (429)	100	CM004 (429)
100	CE026 (744)	100	CN039 (744)	100	CZ030 (744)	100	
100	E040 (675)	100	N062 and N070 (675)	100, 99.6, 99.1, 99.1	Z035, Z041, Z046, and Z058 (675)	100	
100	CE029 (240)					100	CM007 (240)
100	CE030 (1,296)	100	CN050 (1,296)			100	CM008 (1032)
98.4	CE030 (1,296)	98.4	CN050 (1,296)			100	CM009 (375)
100	<i>orfX</i> (480)	100	<i>orfX</i> (480)	100	<i>orfX</i> (480)	100	CM010 (480)
70.3	E026 (1,770)	80.7	N031 (1,794)	72.1	Z004 (1,569)		
75.2	<i>ccrA1</i> (1,350)	96.2	<i>ccrA2</i> (1,350)	73.5	<i>ccrA3</i> (1,350)		
80.6	<i>ccrB1</i> (1,152)	98.2	<i>ccrB2</i> (1,629)	87.1	<i>ccrB3</i> (1,629)		
85.3	E031 (351)	88.8	N041 (351)	5.3	Z011 (351)		
85.4	E032 (327)	89.2	N042 (312)	4.2	Z013 (396)		
97.6	E033 (510)	92.3	N043 (318)	61.3	Z014 (522)		
100	E034 (1,659)						
100	Δ <i>mecRI</i> _{NCTC10442} (987)	100	<i>mecRI</i> _{N315} (1,758)	100	Δ <i>mecRI</i> _{85/2082} (114)		
99.9	<i>mecA</i> _{NCTC10442} (2,007)	100	<i>mecA</i> _{N315} (2,007)	99.7	<i>mecA</i> _{85/2082} (2,007)		
99.3	CE025 (429)	100	CN038 (429)	100	CZ029 (429)		
100	CE026 (744)	100	CN039 (744)	100	CZ030 (744)		
100	E040 (675)	100	N062 and N070 (675)	100, 99.6, 99.1, 99.1	Z035, Z041, Z046, and Z058 (675)		
100	CE029 (240)						
100	CE030 (1,296)	100	CN050 (1,296)				
98.4	CE030 (1,296)	98.4	CN050 (1,296)				
100	<i>orfX</i> (480)	100	<i>orfX</i> (480)	100	<i>orfX</i> (480)		

named the novel SCCmec elements type IV SCCmec and assigned the designations subtypes IVa and IVb to the individual elements of CA05 and 8/6-3P, respectively, on the basis of their unique nucleotide sequences in the L-C region (Fig. 1).

The BLAST and MOTIF programs failed to assign any biological functions to the hypothetical ORFs in the L-C regions of type IV SCCmec elements (1, 17). With their simple genetic organizations, neither a virulence factor nor antibiotic resistance other than that encoded by *mecA* was found to be encoded by the entire regions of the novel SCCmec elements. This lack of genes encoding resistance to non-β-lactam antibiotics in the type IV SCCmec was consistent with the notable characteristic of C-MRSA; i.e., its susceptibility to various antibiotics except β-lactams (3, 6). In fact, as shown in Table 2,

the two strains were susceptible to all the non-β-lactam antibiotics tested.

The lack of function of type IV SCCmec other than those for the movement of the element (*ccr* genes) and methicillin resistance (*mecA*), together with its small size, may lead to the view that the element has gone through evolutionary refinement as a specific carrier of DNA for methicillin resistance. Furthermore, the lack of superfluous functions may make the element more fit as a mobile genetic element for *S. aureus* in the community than any other type of SCCmec, for the following reasons. A number of ORFs carried by type I and type II SCCmec elements in their long L-C regions are considered unnecessary for the benefit of the host cells that carry the elements (11, 12). Actually, many of them are mutated or

TABLE 2. Antibiotic susceptibility profiles of the two C-MRSA strains in comparison with those of H-MRSA strains

Strain	Type of SCCmec	MIC (mg/liter) ^a							
		Oxacillin	Ampicillin	Ceftizoxime	Imipenem	Erythromycin	Tobramycin	Kanamycin	Tetracycline
CA05	IV	8	32	128	0.125	0.5	0.25	2	0.125
8/6-3P	IV	8	16	128	0.125	0.125	0.125	1	0.125
NCTC 10442	I	256	256	>512	16	0.125	0.125	1	128
N315	II	16	32	16	1	>512	512	>512	0.125
85/2082	III	32	32	>512	0.5	>512	8	512	128
ATCC 29213 ^b		0.25	0.5	4	0.03	0.125	0.25	1	0.125

^a The MICs were determined by the agar plate dilution method of the NCCLS.

^b A methicillin-susceptible *S. aureus* type strain.

partially deleted and do not seem to be active. Moreover, an ORF for type I SCCmec encoding plasmin-sensitive surface protein may even be hazardous to the host cell because it interferes with the fibrinogen- and fibronectin-binding properties of the host cell (23). Although the type III SCCmec has a short L-C region comparable in size to those of type IV SCCmec elements, the size of the element is extremely large (68 kb) because of the accumulation of multiple genes for resistance to various antibiotics and heavy metals (12). The determinants for resistance to multiple antibiotics carried by the previously studied types of SCCmec elements (type II and type III elements) may be suited for the survival of H-MRSA in the hospital environment, where various antibiotics as well as antiseptics provide selective pressure, but their large sizes and potentially hazardous arrays of exogenous genes may not be suited to MRSA strains in the community, where selective advantage would make strains more inclined to have a higher growth rate and to be better able to colonize humans than to have a multidrug resistance phenotype. From this viewpoint, the type IV SCCmec may be one of the fit SCCmec types that can confer β -lactam resistance to community strains of *S. aureus* without greatly compromising their competitiveness among the natural flora of humans. Future analyses of many community-acquired strains will be required to test if this hypothesis holds true.

Nucleotide sequence accession numbers. The subtype a and b type IV SCCmec elements have been deposited in the DDBJ/EMBL/GenBank databases under accession no. AB063172 and AB063173, respectively.

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