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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).

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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 α5 and cLs1 (CA05) or CL2b (8/6-3P) (Fig. 1). Primers α6 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 IS*431mec* 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*, *attBscc*, 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 (DRscc-R) at the right extremities and the other (DRscc-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 *attBscc* 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,

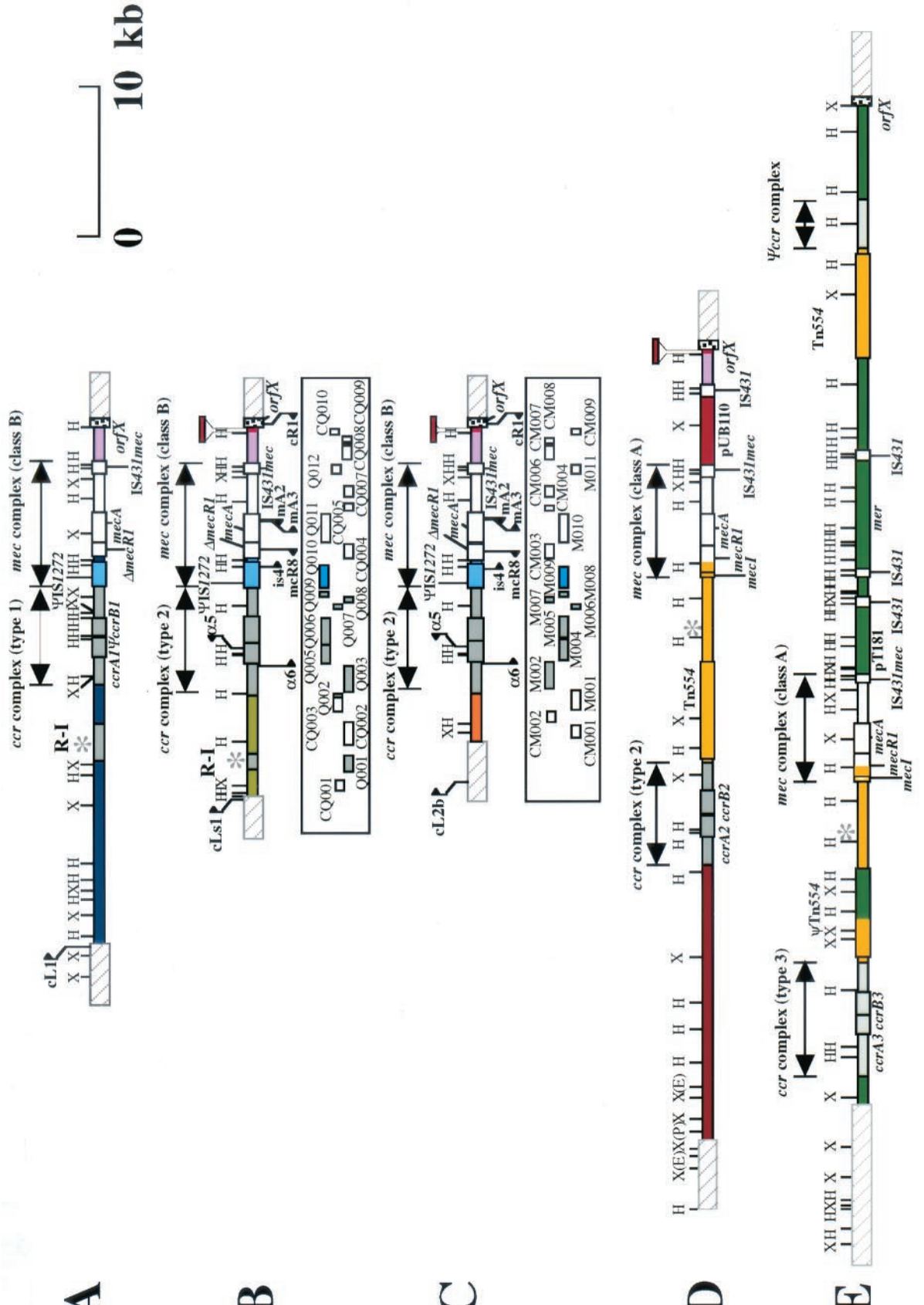
A
B
C
D
E
0
10 kb
ccr complex (type 1)
mec complex (class B)
R-1
ΨIS1272
ccrA1Ψ ccrB1
ΔmecR1
mecA
orfX
IS431mec
ccr complex (type 2)
cLs1
α5
α6
is4
mecR8
mA3
cR1
CQ001
CQ002
CQ003
CQ004
CQ005
CQ006
CQ007
CQ008
CQ009
CQ010
CQ011
CQ012
cL2b
CM001
CM002
CM003
CM004
CM005
CM006
CM007
CM008
CM009
CM010
CM011
M001
M002
M003
M004
M005
M006
M007
M008
M009
M010
ccr complex (type 3)
mec complex (class A)
Ψccr complex
Tn554
ccrA2 ccrB2
ccrA3 ccrB3
pUB110
pT181
IS431
mer
mecI

FIG. 1. Structure of the SCC*mec* elements identified from C-MRSA strains in comparison with the three types of SCC*mec* elements. (A) Type I SCC*mec* carried by NCTC 10442. (B) Type IV SCC*mec* carried by strain CA05 (subtype a). (C) Type IV SCC*mec* carried by strain 8/6-3P (subtype b). (D) Type II SCC*mec* carried by N315. (E) Type III SCC*mec* carried by 85/2082. The ORFs of greater than 200 nucleotides in six possible reading frames of type IV SCC*mec* elements are illustrated in the squares under the bars that represent essential genes and restriction sites for *Hin*dIII 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 SCC*mec* elements with greater than 99% amino acid identities; gray, ORFs or the parts of ORFs that are conserved in four types of SCC*mec* with amino acid identities of 46 to 98%; magenta, ORFs or the parts of ORFs that are common to type I and type II SCC*mec* elements; yellow, ORFs or the parts of ORFs that are common to type II and type III SCC*mec* elements; blue, ORFs or the parts of ORFs that are unique to type I SCC*mec*; red, ORFs or the parts of ORFs that are unique to type II SCC*mec*; green, ORFs or the parts of ORFs that are unique to type III SCC*mec*, light green, ORFs or the parts of ORFs that are unique to type IVa SCC*mec*; and orange, ORFs or the parts of ORFs that are unique to type IVb SCC*mec*. The locations of primers used for amplification of the type IV SCC*mec* are indicated by arrows: they are primers α5 (5′-TGTTAAGTATATTGCACTTTATGATTCAATGCCT-3′), cLs1 (5′-TGCCAATCACAGTTCAATCAATT-3′), α6 (5′-ATTAGCCGATTTGGTAATTGAA-3′), mCR8 (5′-ATATTCCCGTATGAAAAACAGGACTTGAACTTGCA-3′), and CL2b (ATAT TCCCGATAGAAAAACAGGACTTGAACTTGCA) and previously described primers is4, mA2, mA3, and cR1 (11, 12, 14). The entire nucleotide sequences of the type IV SCC*mec* elements are available in the DDBJ/EMBL/GenBank databases under accession no. AB063172 (subtype a) and AB063173 (subtype b). H, *Hin*dIII, X, *Xba*I; E, *Eco*RI; P, *Pst1*.

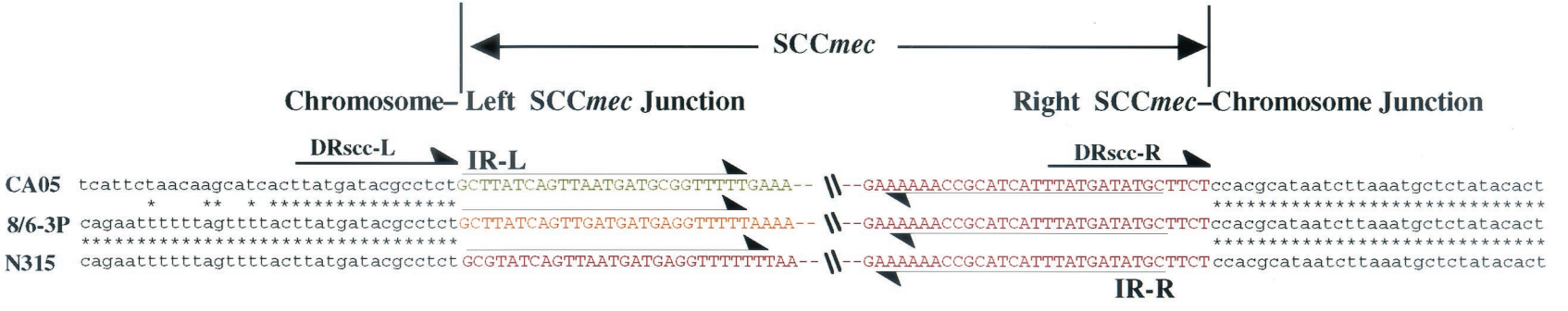

FIG. 2. Chromosome-SCC*mec* junction sequences of type IV SCC*mec*. The nucleotide sequences around the left and right boundaries of the SCC*mec* elements of CA05 and 8/6-3P are aligned with those of type II SCC*mec*. Thin arrows indicate inverted repeats IR-L and IR-R at both extremities of SCC*mec*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

TABLE 1. ORFs in SCC*mec*'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 SCC*mec* 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 (*ccrA2.1*)	Cassette chromosome recombinase A
Q006	11259–12887	1,629 (*ccrB2.1*)	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 (Δ*mecR1*)	Truncated signal transducer protein MecR1
Q011	17646–19652	2,007 (*mecA*)	PBP 2′
CQ005	Complement (19698–20126)	429	Conserved hypothetical protein
CQ007	Complement (20223–20966)	744 (*ugpQ*)	Glycerophosphoryldiester phosphodiesterase
Q012	22268–22942	675 *tnp*	Transposase for insertion sequence-like element IS*431me*[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 SCC*mec* 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 (*ccrA2.2*)	Cassette chromosome recombinase A
M005	7256–8884	1,629 (*ccrB2.2*)	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 IS*1272*
CM003	Complement (12557–13543)	987 (Δ*mecR1*)	Truncated signal transducer protein MecR1
M010	13643–15649	2,007 (*mecA*)	PBP 2′
CM004	Complement (15695–16123)	429	Conserved hypothetical protein
CM006	Complement (16220–16963)	744 (*ugpQ*)	Glycerophosphoryldiester phosphodiesterase
M011	18265–18939	675 *tnp*	Transposase for insertion sequence-like element IS*431me*[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 (IS*1272*-Δ*mecR1-mecA*-IS*431*), 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 SCC*mec*		Homology to ORFs of type II SCC*mec*		Homology to ORFs of type III SCC*mec*		Homology to ORFs of type IV SCC*mec* 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)		
				77.8	CZ002 (1,068)		
46.2	E025 (297)	46	N030 (297)	45.7	Z003 (288)		
77.3	E026 (1,770)	85.3	N031 (1,794)	66.3	Z004 (1,569)	82.4	M002 (1791)
75	*ccrA1* (1,350)	97.6	*ccrA2* (1,350)	72.4	*ccrA3* (1,350)	98.2	M004 (1350)
72.4	**ccrB1* (1,152)	97.4	*ccrB2* (1,629)	87.5	*ccrB3* (1,629)	99.3	M005 (1629)
85.3	E031 (351)	88.8	N041 (351)	51.3	Z011 (351)	100	M006 (351)
85.4	E032 (327)	89.2	N042 (312)	47.2	Z013 (396)	100	M007 (312)
97.6	E033 (510)	92.7	N043 (318)	61.3	Z014 (522)	100	M008 (510)
100	E034 (1,659)					100	M009 (1659)
100	$\Delta mecR1_{NCTC10442}$ (987)	100	$mecR1_{N315}$ (1,758)	100	$\Delta mecR1_{85/2082}$ (114)	100	
99.9	$mecA_{NCTC10442}$ (2,007)	100	$mecA_{N315}$ (2,007)	99.7	$mecA_{85/2082}$ (2007)	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	*orfX* (480)	100	*orfX* (480)	100	*orfX* (480)	100	CM010 (480)
70.3	E026 (1,770)	80.7	N031 (1,794)	72.1	Z004 (1,569)		
75.2	*ccrA1* (1,350)	96.2	*ccrA2* (1,350)	73.5	*ccrA3* (1,350)		
80.6	*ccrB1* (1,152)	98.2	*ccrB2* (1,629)	87.1	*ccrB3* (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	$\Delta mecR1_{NCTC10442}$ (987)	100	$mecR1_{N315}$ (1,758)	100	$\Delta mecR1_{85/2082}$ (114)		
99.9	$mecA_{NCTC10442}$ (2,007)	100	$mecA_{N315}$ (2,007)	99.7	$mecA_{85/2082}$ (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	*orfX* (480)	100	*orfX* (480)	100	*orfX* (480)		

named the novel SCC*mec* elements type IV SCC*mec* 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 SCC*mec* 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 SCC*mec* elements. This lack of genes encoding resistance to non-β-lactam antibiotics in the type IV SCC*mec* 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 SCC*mec* 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 SCC*mec*, for the following reasons. A number of ORFs carried by type I and type II SCC*mec* 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 SCC*mec*	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 SCC*mec* 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 SCC*mec* has a short L-C region comparable in size to those of type IV SCC*mec* 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 SCC*mec* 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 SCC*mec* may be one of the fit SCC*mec* 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 SCC*mec* elements have been deposited in the DDBJ/EMBL/GenBank databases under accession no. AB063172 and AB063173, respectively.

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