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Dissemination of New Methicillin-Resistant *Staphylococcus aureus* Clones in the Community

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Multiple methicillin-resistant *Staphylococcus aureus* (MRSA) clones carrying type IV staphylococcal cassette chromosome *mec* were identified in the community-acquired MRSA strains of both the United States and Australia. They multiplied much faster than health-care-associated MRSA and were resistant to fewer non-beta-lactam antibiotics. They seem to have been derived from more diverse *S. aureus* populations than health-care-associated MRSA strains.

Methicillin-resistant *Staphylococcus aureus* (MRSA), besides having established itself as a major hospital pathogen, is now beginning to prevail in the wider community as well (1, 3–5). However, we do not know if the subgroup of MRSA designated community-acquired MRSA (C-MRSA) share a common origin of derivation with the other subgroup of MRSA in hospitals, namely the health-care-associated MRSA (H-MRSA). The majority of H-MRSA strains carry one of the three types of staphylococcal cassette chromosome *mec* (SCC*mec*) as the methicillin resistance determinant on their chromosomes (19, 22). However, members of our group have recently identified a novel SCC*mec*, designated type IV, in the C-MRSA strains isolated at a Chicago children's hospital (23). This raised a possibility that C-MRSA might have an origin of derivation distinct from that of H-MRSA, and type-IV SCC*mec* could be its unique genetic marker (14). To further test this view, we now analyzed 23 well-characterized C-MRSA strains (2–4, 24–26, 28) whose sources of isolation were not associated with risk factors for H-MRSA infection (e.g., recent hospitalization, recent surgery, residence in a long-term care facility, drug use, etc.) (7, 11) and 12 Australian MRSA strains designated non-multiresistant oxacillin-resistant *S. aureus* (NORSA) (9) and compared them with the representative H-MRSA strains. NORSA strains, though frequently isolated in hospitals, are considered to be the descendants of C-MRSA strains in Australia (10).

Table 1 shows that the majority of C-MRSA strains were susceptible to most of the non-beta-lactam antibiotics, as NORSA strains are by definition (9). Although the non-multiresistant nature of C-MRSA has been well recognized as a characteristic of C-MRSA (16), this was not without exception: strain 01083 was resistant to four non-beta-lactam antibiotics (Table 1). This indicates that though it is a rare occurrence, C-MRSA strains may also acquire resistance to non-beta-lactam antibiotics, presumably through exposure to the antibiotics.

Table 1 also shows that C-MRSA/NORSA strains had relatively lower levels of oxacillin and imipenem resistance than H-MRSA strains (with the exceptions of N315 and 85/2082) (20). This indicated that they had the heterogeneous methicillin resistance phenotype, which was confirmed by population analysis (Fig. 1). MW2, a representative C-MRSA strain (2), possessed typical heterogeneous subpopulations of resistant cells, whereas the "truly" (i.e., *mecA*-negative) methicillin-susceptible strain 476, the putative progenitor strain of MW2 (see below), did not have the resistant subpopulations. Mu3, a typical H-MRSA strain, on the other hand, had a distinct pattern of resistance called homogeneous methicillin resistance. This implied that unlike H-MRSA strains, C-MRSA strains were not selected out by the exposure to these potent beta-lactam antibiotics used in the hospital, testifying further to their predominant propagation occurring in the community.

C-MRSA/NORSA strains grew significantly faster than H-MRSA strains: the mean doubling times (8) of the former group of strains were 28.79 ± 7.09 and 28.24 ± 2.48 min, respectively, whereas that for the latter was 38.81 ± 7.01 min (see Table 1). The difference was statistically significant (P value of <0.0001 by t test). This high growth rate may be a prerequisite in the absence of antibiotics for C-MRSA to achieve successful colonization of humans by outcompeting the numerous bacterial species in the environment.

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TABLE 1. Genotyping and antibiogram of tested MRSA strains

Category[a]	Source[b]	Strain name	Coagulase isotype	MIC (mg/liter) of[c]:									SCC*mec* type[d]	PFGE pattern	MLST		*luk-PV*[g]	Doubling time (min)[h]	Reference(s)
				ERY	KAN	TOB	GEN	TET	NOR	OXA	CZX	IMP			ST, allelic profile[e]	CC[f]			
C	MN, US	C1998000370	7	0.5	8	0.5	0.5	**32**	1	**4**	**512**	0.25	IVa	J4	1, 1-1-1-1-1-1-1	1	+	27.89	3, 24
C	ND, US	C1999000529	7	0.5	4	0.5	0.25	0.5	1	**8**	**>512**	0.5	IVa	J4	1, 1-1-1-1-1-1-1	1	+	26.38	3, 24
C	MN, US	C1999000193	7	0.5	8	0.5	0.5	0.5	1	**4**	**>512**	0.25	IVa	J3	1, 1-1-1-1-1-1-1	1	+	26.73	3, 24
C	ND, US	MW2	7	0.5	4	0.25	0.25	0.5	1	**8**	**>512**	0.5	IVa	J4	1, 1-1-1-1-1-1-1	1	+	28.67	2, 3, 24
C	MN, US	C2001001201	7	**>512**	8	0.25	0.25	0.5	1	**8**	**>512**	0.5	IVa	J4	1, 1-1-1-1-1-1-1	1	+	27.4	3, 24
C	MN, US	C2001000101	7	0.5	8	0.5	0.5	0.5	1	**4**	**>512**	0.25	IVa	J4	1, 1-1-1-1-1-1-1	1	+	27.25	3, 24
C	MN, US	C200100818	7	**>512**	8	0.5	0.25	0.5	1	**16**	**>512**	0.25	IVa	J5	1, 1-1-1-1-1-1-1	1	+	27.72	3, 24
C	Woo, AU	A80 3355	4	0.5	4	0.25	0.25	0.5	2	**16**	**>512**	0.125	IVa	H1	30, 2-2-2-2-6-3-2	30	+	26.22	25
C	Woo, AU	A82 3549	4	0.5	2	0.25	0.25	0.5	2	**16**	**>512**	0.125	IVa	H3	30, 2-2-2-2-6-3-2	30	+	28.09	25
C	Woo, AU	A83 0528	4	0.5	4	0.25	0.25	0.5	2	**16**	**>512**	≤0.063	IVa	H1	30, 2-2-2-2-6-3-2	30	+	27.08	25
C	Woo, AU	B82 6559	4	0.5	2	0.25	0.25	0.5	2	**16**	**512**	≤0.063	IVa	H1	30, 2-2-2-2-6-3-2	30	+	27.57	25
C	Woo, AU	D82 1552	4	0.5	2	0.25	0.25	0.5	2	**16**	**>512**	0.125	IVa	H1	30, 2-2-2-2-6-3-2	30	+	61.03	25
C	Woo, AU	E80 2537	4	0.5	4	0.25	0.25	0.5	2	**8**	**>512**	≤0.063	IVa	H2	30, 2-2-2-2-6-3-2	30	+	26.97	25
C	Woo, AU	F81 0539	4	0.5	4	0.25	0.25	0.5	2	**16**	**>512**	≤0.063	IVa	H1	30, 2-2-2-2-6-3-2	30	+	26.44	25
C	Woo, AU	180 2552	4	0.5	2	0.25	0.25	0.5	2	**16**	**512**	0.125	IVa	H3	30, 2-2-2-2-6-3-2	30	+	26.86	25
C	Per, AU[i]	M9N	3	**>512**	4	0.25	0.25	0.5	2	**16**	**512**	0.125	IVa	I1	78, 22-1-14-23-12-53-31	298	−	26.75	26
C	Per, AU[i]	M33T	3	**>512**	4	0.25	0.25	0.5	2	**16**	**512**	0.125	IVa	I2	78, 22-1-14-23-12-53-31	298	−	26.44	26
C	Per, AU[i]	W1S	1	0.5	2	0.5	0.25	0.5	0.5	2	**32**	0.063	New	E2	45, 10-14-8-6-10-3-2	45	−	27.74	26
C	Per, AU[i]	C7N	7	**>512**	4	0.5	0.5	0.5	2	**16**	**512**	0.25	IVa	J6	1, 1-1-1-1-1-1-1	1	−	30.26	26
C	TN, US[j]	00215	7	0.25	4	0.5	0.25	0.5	0.5	**64**	**>512**	2	IVa	E1	45, 10-14-8-6-10-3-2	45	−	27.71	28
C	MS, US[k]	01083	3	**64**	**>512**	0.5	0.5	**32**	**16**	**8**	**512**	0.125	IVa	A3	8, 3-3-1-1-4-4-3	8	+	27.78	4
C	MS, US[k]	01093	5	**16**	**512**	0.5	0.5	0.5	2	**16**	**>512**	0.125	IVa	F	72, 1-4-1-8-4-4-3	8	−	25.94	4
C	MS, US[k]	01102	3	0.25	4	0.5	0.5	0.5	1	**8**	**>512**	0.25	IVb	A2	8, 3-3-1-1-4-4-3	8	+	28.29	4
N	Ade, AU	81 0342	3	0.25	4	0.5	0.5	0.5	**8**	2	16	≤0.063	New	A1	8, 3-3-1-1-4-4-3	8	−	26.86	This study
N	Ade, AU	91 2572	7	0.5	4	0.25	0.25	0.5	1	**4**	**>512**	0.25	IVa	J7	1, 1-1-1-1-1-1-1	1	−	27.15	This study
N	Ade, AU	91 2619	7	0.5	8	0.5	0.5	0.5	2	**64**	**>512**	2	IVa	J7	76, 1-63-1-1-1-1-1	1	−	27.47	This study
N	Ade, AU	WCH379	7	0.5	2	0.25	0.5	0.5	1	**16**	**>512**	4	IVa	J8	1, 1-1-1-1-1-1-1	1	−	33.31	This study
N	Ade, AU	91 2574	7	0.25	1	0.25	0.25	0.5	**128**	**8**	**256**	≤0.063	New	G	22, 7-6-1-5-8-8-6	22	−	29.52	This study
N	Ade, AU	SAP260	2	**>512**	4	0.5	0.25	0.5	0.5	**4**	**>512**	0.25	IVa	B	73, 1-4-27-4-12-1-10	5	−	26.64	This study
N	Per, AU	81 0937	7	0.5	2	0.25	0.5	0.5	1	**4**	**>512**	0.5	IVa	J1	1, 1-1-1-1-1-1-1	1	−	25.29	This study
N	Per, AU	91 2125	7	0.5	4	0.25	0.5	0.5	0.5	**16**	**>512**	0.5	IVa	J2	1, 1-1-1-1-1-1-1	1	−	30.25	This study
N	Per, AU	91 1703	3	0.25	2	0.125	0.25	**8**	1	**16**	**512**	0.125	IVa	A2	8, 3-3-1-1-4-4-3	8	−	32.0	This study
N	Bri, AU	81 1238	7	0.5	2	0.25	0.5	0.5	1	**8**	**>512**	0.5	IVa	J1	1, 1-1-1-1-1-1-1	1	−	26.06	This study
N	Bri, AU	91 2666	7	0.5	2	0.5	0.25	0.5	0.5	2	**512**	0.125	IVa	J2	1, 1-1-1-1-1-1-1	1	−	26.65	This study
N	Dar, AU	SAP411	6	≤0.125	4	0.25	0.5	**32**	1	**4**	**128**	0.125	IVa	N	75, 36-3-43-34-39-52-49	S	−	27.68	This study
H	Ade, AU	81 0508	4	**>512**	**256**	**256**	0.5	0.25	**512**	**512**	**>512**	**128**	II	M2	36, 2-2-2-2-3-3-2	30	−	32.22	This study
H	Ade, AU	91 2231	4	**>512**	2	0.25	0.5	**32**	**32**	**128**	**>512**	**16**	III	K4	239, 2-3-1-1-4-4-3	8	−	35.8	This study
H	Bri, AU	91 1573	4	**>512**	**128**	**64**	**8**	**16**	1	**64**	**>512**	4	III	K2	239, 2-3-1-1-4-4-3	8	−	41.82	This study
H	Bri, AU	91 1575	4	**>512**	**512**	16	**32**	1	**32**	**256**	**>512**	**32**	III	K6	239, 2-3-1-1-4-4-3	8	−	50.46	This study
H	Bri, AU	91 2145	4	**>512**	**512**	16	**32**	**32**	**32**	**64**	**>512**	**16**	III	K1	239, 2-3-1-1-4-4-3	8	−	50.64	This study
H	Dar, AU	SAP344	4	**>512**	4	0.25	0.5	**32**	**32**	**256**	**>512**	**32**	III	K3	239, 2-3-1-1-4-4-3	8	−	34.51	This study
H	Per, AU	91 2118	4	**>512**	**512**	16	**32**	**32**	**256**	**512**	**>512**	**64**	III	L	239, 2-3-1-1-4-4-3	8	−	38.45	This study
H	UK	NCTC10442	3	0.125	1	0.125	0.5	**128**	1	**256**	**>512**	**16**	I	D	250, 3-3-1-1-4-4-16	8	−	36.44	19
H	JP	N315	2	**>512**	**>512**	**512**	0.5	0.125	2	**16**	16	1	II	C	5, 1-4-1-4-12-1-10	5	−	34.28	19
H	NZ	85/2082	4	**512**	**>512**	8	**64**	**128**	2	**32**	**>512**	0.5	III	K5	239, 2-3-1-1-4-4-3	8	−	43.53	19
H	UK	Strain 252[l]	4	**>512**	**128**	**128**	0.25	0.5	**>512**	**512**	**>512**	**64**	II	M1	36, 2-2-2-2-3-3-2	30	−	29.79	20, —[m]
H	UK	MSSA476	7	0.5	4	0.5	0.5	1	1	0.5	8	≤0.063		J1	1, 1-1-1-1-1-1-1	1	−	27.66	—[m]

[a] C, C-MRSA; N, NORSA; H, H-MRSA.

[b] MN, Minnesota; US, United States; ND, North Dakota; Woo, Wooloongabba, Australia, AU, Australia; Per, Perth, Australia; TN, Tennessee; MS, Mississippi; Ade, Adelaide, Australia; Bri, Brisbane, Australia; Dar, Darwin, Australia; UK, United Kingdom; JP, Japan; NZ, New Zealand.

[c] MICs were determined by the NCCLS-based plate dilution method. Antibiotics: ERY, erythromycin; KAN, kanamycin; TOB, tobramycin; GEN, gentamicin; TET, tetracycline; NOR, norfloxacin; OXA, oxacillin; CZX, ceftizoxime; IMP, imipenem. Values in bold signify resistance to these antibiotics.

[d] New, new type of SCC*mec* possessing class C2 *mec* gene complex (see Fig. 2).

[e] ST, sequence type.

[f] Clonal complex, based on BURST (based upon related sequence types). S, singleton (not assigned to any clonal complex).

[g] *luk-PV* genes encode Panton-Valentin leucocidin proteins.

[h] Doubling time during exponential growth phase (optical density of 0.05 to ~1.0 at 660 nm) measured by using TN-2612 (Advantec Toyo Kaisha, Ltd., Tokyo, Japan) as previously described (8).

[i] Isolated from aborigine.

[j] Isolated from food-poisoning strain.

[k] Isolated from state prison.

[l] E-MRSA16.

[m] —, http://www.sanger.ac.uk/Projects/S.aureus/.

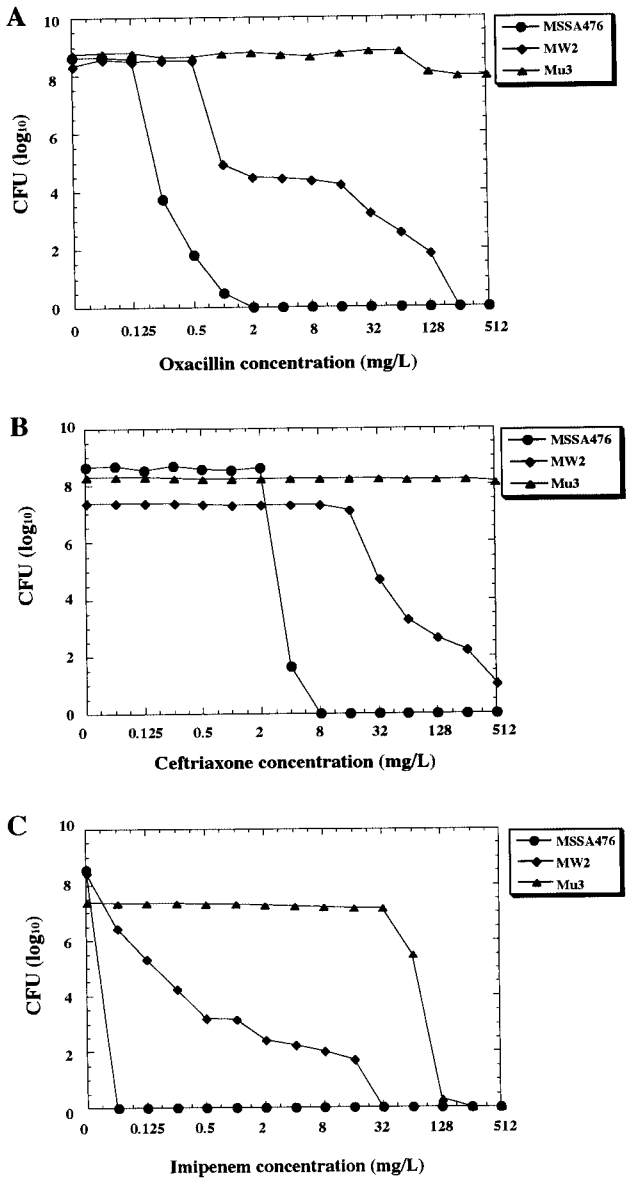

FIG. 1. C-MRSA strain shows heterogeneous phenotypic expression of methicillin resistance. Analysis of resistant subpopulations of the C-MRSA strain MW2, the related MSSA strain 476, and strain Mu3, with heterogeneous resistance to vancomycin, was performed with oxacillin (A), ceftriaxone (B), and imipenem (C) as described previously (13). Ceftriaxone was the antibiotic used in vain to treat the patient infected with MW2 (3). MW2 is an American Midwest MRSA strain representing the major C-MRSA (see the text). Strain 476 is an MSSA strain sharing its MLST allotype with MW2 (see Table 1). Mu3 is a representative H-MRSA strain with heterogeneous resistance to vancomycin (13). It is noted that MW2 contains subpopulations resistant to each of the three beta-lactam antibiotics.

The MRSA genotype is the sum of the SCC*mec* type and the type of its recipient chromosome (12). First, by using multiple locus sequence typing (MLST), we identified the chromosome genotype of the test strains. Enright et al. reported that 356 of 359 MRSA strains from 20 countries were classified into only five clonal complexes (CCs), CC5, CC8, CC22, CC30, and CC45, with the rest, three strains, possessing sequence types (STs) of rare occurrence (6). All the 11 H-MRSA strains used in this study were reasonably classified into three of these five CCs (Table 1). However, 35 C-MRSA/NORSA strains possessed 10 different STs that constituted one singleton (ST75) and seven CCs that, surprisingly, included all five H-MRSA CCs described above (Table 1).

Among the seven C-MRSA CCs, especially notable was CC1, which contained the internationally dominant C-MRSA strains; eight U.S. strains represented by MW2 and six Australian strains belonged to this clonal complex. Remarkably, no H-MRSA strains belonged to this complex (6). Curiously, a highly virulent methicillin-susceptible *S. aureus* (MSSA) strain, 476, whose whole genome sequence has been determined, belongs to this complex (http://www.mlst.net/new/index.htm). MSSA 476 and two NORSA strains belonging to CC1 even shared an identical pulsed-field gel electrophoresis (PFGE) pattern (Table 1). Detailed comparison revealed that the only significant difference between the two chromosomes was the presence of type IV SCC*mec* in MW2, which indicated that strain 476 represented the progenitor MSSA strain from which MW2 was generated by acquiring type IV SCC*mec*.

The pattern of clonal distribution of the 35 C-MRSA/NORSA strains was statistically distinct from that of 359 MRSA strains analyzed in a previous study plus 11 H-MRSA strains used in this study (P value of <0.000001 by Fisher's exact test). This clearly indicated that distinct clonal populations were successfully propagated as C-MRSA/NORSA and H-MRSA, presumably through different selective pressures exerted on them, e.g., fast-growing *S. aureus* or *S. epidermidis* strains for the former and exposure to multiple antibiotics for the latter.

The MLST data, despite the small number of tested strains, indicated that C-MRSA/NORSA strains were generated from *S. aureus* clones of much more diverse genetic backgrounds than expected. This was also supported by PFGE analysis (Table 1), which showed that the C-MRSA/NORSA strains were classified into nine unrelated groups according to the criteria described by Tenover et al. (27). Moreover, these strains consisted of producers of as many as seven coagulase isotypes (Table 1). Since only eight coagulase isotypes are known among *S. aureus* strains isolated from various sources (18), this also supported the view that C-MRSA/NORSA represents diverse *S. aureus* genomes as the origin of derivation.

Next, we determined SCC*mec* types by PCR typing of the *mec* gene complex and *ccr* gene complex as described previously (19, 21). Table 2 and Fig. 2 show the nucleotide sequences and locations of the primers used (15, 19, 21). In contrast to the heterogeneity of C-MRSA/NORSA chromosomes demonstrated above, all except for three strains harbored type IV SCC*mec*, and the remaining three harbored a novel SCC*mec* carrying the class C2 *mec* gene complex (21) (Fig. 2). None of the C-MRSA/NORSA strains possessed any of the three types of SCC*mec* which the majority of epidemic H-MRSA strains possess (19).

It is not clear at this moment why type IV SCC*mec* is prev-

TABLE 2. Primer sets used for identifying SCC*mec*

Primer (previous name) for detection of:	Nucleotide sequence	Expected size of product (gene[s] reactive to the primer)	Reference
ccr gene complex[a]			
βc (β2)	5′-ATTGCCTTGATAATAGCCITCT-3′	(all types of *ccrB*)	19
αc	5′-ATCTATTTCAAAAATGAACCA-3′	560 bp, βc (all types of *ccrA*)	This study
α1 (α2)	5′-AACCTATATCATCAATCAGTACGT-3′	700 bp, βc (type 1 *ccrA*)	19
α2 (α3)	5′-TAAAGGCATCAATGCACAAACACT-3′	1 kbp, βc (type 2 *ccrA*)	19
α3 (α4)	5′-AGCTCAAAAGCAAGCAATAGAAT-3′	1.6 kbp, βc (type 3 *ccrA*)	19
mec gene complex (all types)			
mecR1 (MS domain)			
mcR4	5′-GTCGTTCATTAAGATATGACG-3′	(*mecR1*—MS domain)	This study
mcR3	5′-GTCTCCACGTTAATTCCATT-3′	310 bp, mcR4 (*mecR1*—MS domain)	21
mecA			
mA1	5′-TGCTATCCACCCTCAAACAGG-3′	(*mecA*)	15
mA2	5′-AACGTTGTAACCACCCCAAGA-3′	200 bp, mA1 (*mecA*)	15
mecA -IS*431mec*			
IS2 (iS-2)	5′-TGAGGTTATTCAGATATTTCGATGT-3′	4 kbp, mA1 (IS*431mec*)	21
Class C *mec* gene complex			
mA2	5′-AACGTTGTAACCACCCCAAGA-3′	<3 kbp, IS*2* (*mecA*)	21
Class B *mec* gene complex			
IS5	5′-AACGCCACTCATAACATATGGAA-3′	(IS*1272*)	This study
mA6	5′-TATACCAAACCCGACAAC-3′	2 kbp, IS*5* (*mecA*)	21
Class A *mec* gene complex			
mecI			
mI4	5′-CAAGTGAATTGAAACCGCCT-3′	(*mecI*)	This study
mI3	5′-CAAAAGGACTGGACTGGAGTCCAAA-3′	180 bp, mI4 (*mecI*)	This study
mecR1 (PB domain)			
mcR2	5′-CGCTCAGAAATTTGTTGTGC-3′	(*mecR1*—PB domain)	21
mcR5	5′-CAGGGAATGAAAATTATTGGA-3′	320 bp, mcR2 (*mecR1*—PB domain)	This study
SCC*mec* subtype IVa			
4a1	5′-TTTGAATGCCCTCCATGAATAAAAT-3′	(J1 region of IVa)	This study
4a2	5′-AGAAAAGATAGAAGTTCGAAAGA-3′	450 bp, 4a1 (J1 region of IVa)	This study
SCC*mec* subtype IVb			
4b1	5′-AGTACATTTTATCTTTGCGTA-3′	(J1 region of IVb)	This study
4b2	5′-AGTCATCTTCAATATCGAGAAAGTA-3′	1 kbp, 4b1 (J1 region of IVb)	This study

[a] *ccr* type is determined by PCR using primer βc (the common primer for three types of *ccrB*) and either one of the three types of *ccrA*, α1 (*ccrA1*), α2 (*ccrA2*), and α3 (*ccrA3*). This typing actually reflects the allotype of *ccrA*.

alent in C-MRSA/NORSA strains. However, type IV SCC*mec* is short (21 to 25 kb) compared to the three SCC*mec*s prevalent in H-MRSA strains (34 to 67 kb) and lacks any antibiotic resistance genes other than *mecA* (23) (Fig. 2). This evidently corresponds to the non-multiresistant nature of C-MRSA/NORSA and may alleviate the fitness cost paid by H-MRSA strains carrying big SCC*mec*s with multiple-drug resistance determinants.

Although we need to explore further the reason why type IV SCC*mec* is prevalent in C-MRSA strains, it seems clear that we are witnessing the emergence and expansion of new MRSA clones in the community. These clones are different from any of the major H-MRSA clones in the world that we have identified by using SCC*mec* typing and ribotyping combinations (12, 17). In this study we realized that the antibiogram is not completely reliable in discriminating C-

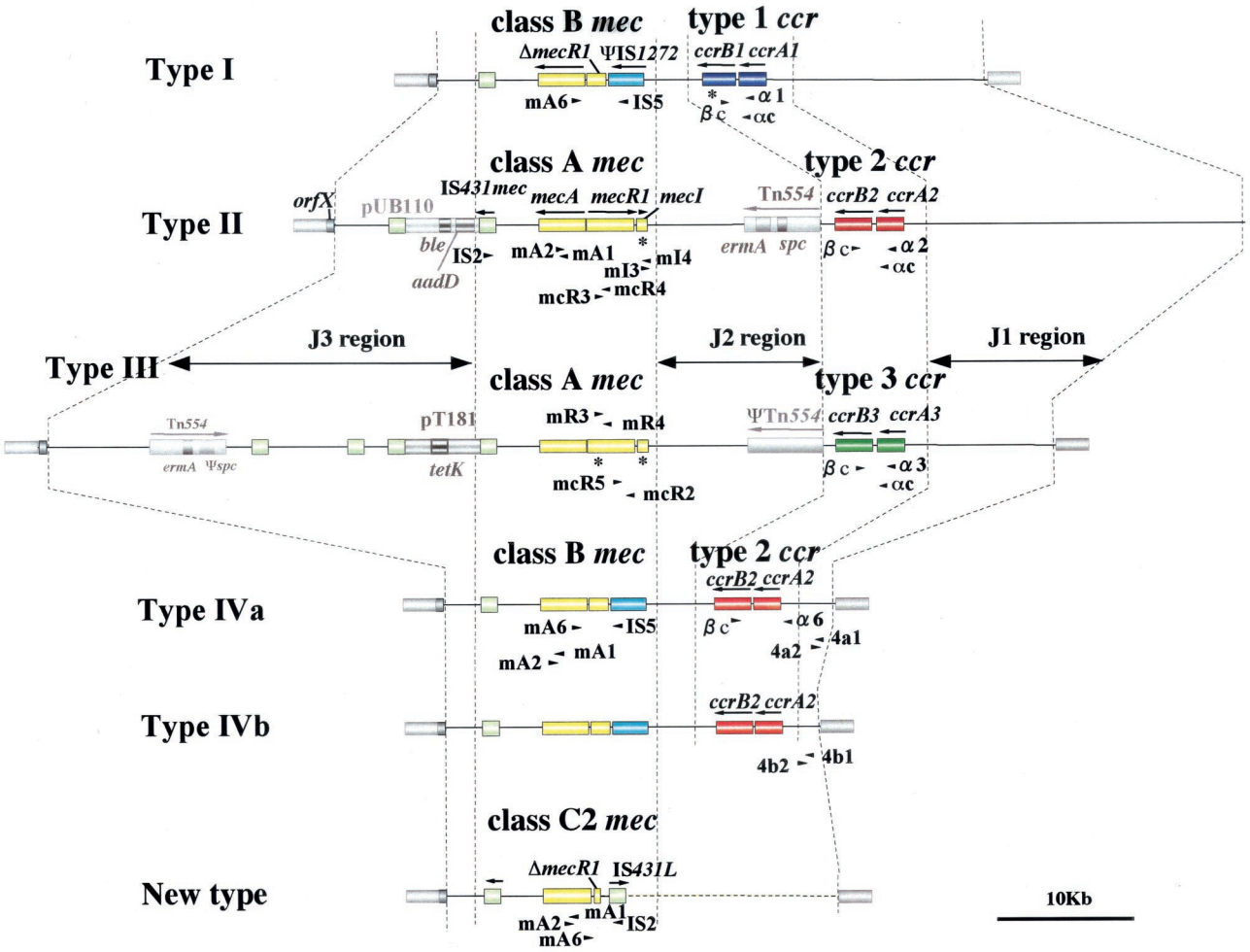

FIG. 2. Illustrative representation of various types of SCC*mec*. SCC*mec* type is defined by the combination of the type of *ccr* gene complex and the class of *mec* gene complex. Type I SCC*mec* is defined by the combination of type 1 *ccr* and class B *mec* (IS*1272*-Δ*mecR1-mecA*); type II is defined by type 2 *ccr* and class A *mec* (*mecI-mecR1-mecA*); type III is defined by type 3 *ccr* and class A *mec*; and type IV is defined by type 2 *ccr* and class B *mec*. Type IV SCC*mec* is further classified into subtypes (type IVa and type IVb) based on the sequence difference in the J1 region of SCC*mec* (J stands for "junkyard"). Positions of primers used in this study to identify and type SCC*mec* are shown (see Table 2 for the nucleotide sequence of each primer). The allelic class of *mec* gene complex is determined by PCR detection of the presence or absence of IS*1272*, *mecI*, and *mecR1* in two domains (PB, penicillin-binding domain; and MS, membrane-spanning domain), *mecA,* and IS*431mec*. PCR amplification was performed using 2.5 U of Ex *Taq* (Takara Shuzo Co., Ltd., Kyoto, Japan) in 50 μl of reaction mixture. Thermal cycling was set at 30 cycles (30 s for denaturation at 94°C, 1 min for annealing at 50°C, and 2 min for elongation at 72°C) using the Gene Amp PCR system 9600 (Perkin-Elmer, Wellesley, Mass.). For the detection of *mecA*-IS*431mec*, a long-range PCR method was used, set at 10 cycles (15 s for denaturation at 94°C, 30 s for annealing at 50°C, and 8 min for elongation at 68°C) followed by 20 cycles (15 s for denaturation at 94°C, 30 s for annealing at 50°C, and 12 min for elongation at 68°C). Note that this study identified a new type of SCC*mec* for three C-MRSA strains that carried the class C2 *mec* gene complex (21). The sequencing of the entire SCC*mec* is now ongoing.

MRSA from H-MRSA, nor is the phenotypic expression of methicillin resistance. Even epidemiological information is not sufficient, since, for example, many C-MRSA strains have been carried in Australian hospitals (29). Therefore, no reliable judgment can be made as to whether the strain isolated in the hospital is H-MRSA or C-MRSA even based on the timing of isolation of the strains after admission to hospital. In this regard, SCC*mec* and MLST typing will become more important in the years to come for discrimination of numerous C-MRSA strains prevailing in both community and hospitals by reference to their ancestral MRSA clones (12).

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