ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Mar. 2006, p. 1001–1012 Vol. 50, No. 3
0066-4804/06/$08.00+0 doi:10.1128/AAC.50.3.1001–1012.2006

Staphylococcal Cassette Chromosome *mec* (SCC*mec*) Typing of Methicillin-Resistant *Staphylococcus aureus* Strains Isolated in 11 Asian Countries: a Proposal for a New Nomenclature for SCC*mec* Elements

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Received 5 August 2005/Returned for modification 16 October 2005/Accepted 31 December 2005

A description of staphylococcal cassette chromosome *mec* (SCC*mec*) elements carried by 615 methicillin-resistant *Staphylococcus aureus* (MRSA) strains isolated in 11 Asian countries is reported, and a novel nomenclatural system based on their structures is proposed. The 615 strains were classified as type 3A (370 strains), type 2A (207 strains), type 2B (32 strains), type 1B (1 strain), and nontypeable (5 strains). The previously reported type III SCC*mec* (DDBJ/EMBL/GenBank accession no. AB037671) carried by the MRSA strain 85/2082 was ascertained to be composed of two SCC elements, type 3A SCC*mec* and SCC*mercury*. PCR analysis indicated that 310 of 370 type 3A SCC*mec* strains carried both SCC elements. These strains were prevalent in eight countries: Thailand, Sri Lanka, Indonesia, Vietnam, Philippines, Saudi Arabia, India, and Singapore. The remaining 60 type 3A SCC*mec* strains differed with respect to the left extremity polymorphism or to the presence of *ccrC*. Among these, two were identified as carrying only type 3A SCC*mec* elements, but their left extremities differed. Type 2A SCC*mec* strains predominated in Korea and Japan, although the frequency of the presence of *ant*(*4′*)-1 gene downstream of *mecA* varied (53% for Korean strains; 93% for Japanese strains). Various SCC*mec* elements were identified in the tested strains, and limited numbers were identified by their multilocus sequence typing genotypes. These data suggest that numerous MRSA clones are disseminated in Asian hospitals, and these consist of minor clones that are presumed to have arisen locally and major clones that are presumed to have been introduced from other countries.

Methicillin, a semisynthetic penicillin that is poorly hydrolyzed by penicillinase, was first used clinically in 1960. Only 1 year later, *Staphylococcus aureus* strains that showed resistance to methicillin were reported (17). Since then, methicillin-resistant *Staphylococcus aureus* (MRSA) strains have appeared in countries worldwide and continue to be one of the most common hospital pathogens (1). It has been shown that methicillin-susceptible *S. aureus* (MSSA) strains become MRSA strains by the acquisition of a staphylococcal cassette chromosome *mec* (SCC*mec*) element carrying the *mecA* gene, which is responsible for methicillin resistance (16, 18). SCC*mec* elements, unique genomic islands that are found in staphylococcal species, have two essential components, the *ccr* gene complex (*ccr*) and the *mec* gene complex (*mec*) (10, 13). The *ccr* gene complex is composed of *ccr* genes and surrounding open reading frames (ORFs), and the *mec* gene complex is composed of the *mecA* gene, regulatory genes, and insertion sequences upstream or downstream of *mecA*. Several *mec* and *ccr* allotypes have been found among SCC*mec* elements, which has led to the following classification (14, 15, 22): type I SCC*mec*, carrying class B *mec* and type 1 *ccr*; type II SCC*mec*, with class A *mec* and type 2 *ccr*; type III SCC*mec*, with class A *mec* and type 3 *ccr*; type IV SCC*mec*, with class B *mec* and type 2 *ccr*; and type V SCC*mec*, class C2 *mec* and type 5 *ccr*.

Recently, MRSA has become increasingly prevalent in community-acquired infections (3, 6, 7, 28, 37). SCC*mec* typing and genotyping have provided strong evidence for the independent origins of health care-associated MRSA and community-acquired MRSA (C-MRSA) (2, 5, 21, 25, 26, 29, 33, 39). While SCC*mec* typing has become essential for the characterization of MRSA clones in epidemiological studies, there are two problematic issues. One concerns assigning of SCC*mec* elements, and the other concerns naming novel elements or variants. We have assigned types of SCC*mec* elements by determining *mec* classes and *ccr* types. On the other hand, Oliveira and Lencastre have developed a multiplex PCR technique for assigning SCC*mec* elements based on the identification of eight loci (A to H) mostly located in the J regions (31). However, we think that the concept that SCC*mec* types can be assigned by determining *mec* class and *ccr* type first has been agreed upon by staphylococcal researchers. Nonetheless, the nomenclature of SCC*mec* types remains problematic. Historically, two type IV SCC*mec* elements have been described. In 2005, two new type II SCC*mec* elements (IIb and IIB) were reported (12, 36). Rules for naming SCC*mec* elements that are

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acceptable to all researchers need to be established. We have developed a new nomenclature for SCC*mec* types, which is an attempt to resolve confusion in the field and to facilitate the identification of novel SCC*mec* elements. This nomenclature is based on the structure of SCC*mec* elements, and it is suitable for describing previously reported SCC*mec* elements as well as those distributed in Asian countries, as far as we can determine. Here, we report on the characteristics of Asian MRSA clones. Our data suggest that MRSA clones may have been generated by the integration of SCC*mec* elements into MSSA clones that have propagated in each country and that MRSA clones in Asian hospitals are more divergent than previously reported (8, 19).

MATERIALS AND METHODS

Bacterial strains. A total of 615 methicillin-resistant *Staphylococcus aureus* strains were analyzed in this study. Specimens were obtained from health care facilities in the following countries from 1998 to 1999 by the Asian Network for Surveillance of Resistant Pathogens: China (Beijing Children's Hospital, Beijing, and Shanghai Children's Hospital, Shanghai), 40 strains; India (Christian Medical College, Vellore), 37 strains; Indonesia (University of Indonesia, Jakarta), 60 strains; Japan, 138 strains; Korea (Samsung Medical Center, Seoul), 105 strains; Philippines (Research Institute for Tropical Medicine, Manila), 15 strains; Saudi Arabia (King Saud University Hospital, Riyadh), 19 strains; Singapore (National University of Singapore, Singapore), 87 strains; Sri Lanka (University of Colombo, Colombo), 29 strains; Thailand (Mahidol University, Bangkok), 49 strains; and Vietnam (University of Medicine and Pharmacy, Ho Chi Minh City), 36 strains.

All strains collected by the Asian Network for Surveillance of Resistant Pathogens program (other than *mecA*-negative strains) were tested, and some were tested by Ko et al. previously (19). *S. aureus* ATCC 25923 was used as a quality control for susceptibility testing.

Five MRSA strains, NCTC10442, N315, 85/2082, CA05, and WIS (WGB8318), were used for the standard strains of type 1A (type I), type 2A (type II), type 3A (type III), type 2B (type IV), and type 5C (type V) SCC*mec* elements.

PCR-based assignment of SCC*mec* elements. Chromosomal DNA was extracted from MRSA strains by the small-scale phenol extraction method and used as templates. PCRs were performed using a thermal cycler Gene Amp 9600 (Perkin-Elmer Cetus Instruments, Emeryville, CA). Reaction mixtures contained 10 ng template DNA, oligonucleotide primers (0.2 μM), 400 μM each deoxynucleoside triphosphate, 1× Ex Taq buffer with magnesium, and 2 U Ex *Taq* polymerase (Takara Bio, Inc., Kyoto, Japan) in a final volume of 50 μl. PCRs consisted of 30 cycles of denaturation (95°C, 30 s), annealing (50°C, 60 s), and extension (72°C, 2 min). Four microliters of each reaction mixture was subjected to agarose gel electrophoresis to detect amplified DNA fragments. The primer sets used for the assignment of SCC*mec* elements are listed in Table 1. The presence of the *mecA* gene was first checked to confirm MRSA status. Class A *mec* strains were identified by amplification of a DNA fragment with one primer specific for the *mecI* gene (mI4) and the other specific for the *mecR1* gene (mcR5). Similarly, class B *mec* and class C *mec* strains were identified by amplification of fragments with primers specific for *mecA* (mA6) and IS*1272* (IS5) or for IS*431* (IS2) and *mecA* (mA2), respectively. The *ccr* gene complexes were typed by multiplex PCR using four primers: βc, a degenerate inosine-containing primer that recognizes *ccrB1*, *ccrB2*, and *ccrB3*; α1, a primer specific for *ccrA1*; α2, a primer specific for *ccrA2*; and α3, a primer specific for *ccrA3*. The *ccr* gene type was determined by the size of the amplified DNA fragment as follows: type 1 *ccr*, 0.7 kb; type 2 *ccr*, 1 kb; and type 3 *ccr*, 1.6 kb (Table 1). Strains for which DNA fragments were not amplified with the above primers were examined for the presence of type 4 *ccr*.

The J region in the SCC*mec* element was further classified into three subregions, J1 (formerly called the L-C region), J2 (the former C-M region), and J3 (the former M-R region). Table 1 shows the list of primers used to distinguish J regions.

PCR-based identification of small type 3A SCC*mec* elements. DNA fragments encompassing the entire SCC*mec* elements of strains JCSC290 and JCSC1716 were amplified by long-range PCR using the Expand High Fidelity PCR system following the manufacturer's recommendations (Roche Diagnostics Co., Indianapolis, IN). Briefly, reactions were performed in a final volume of 50 μl and contained 10 ng template DNA, oligonucleotide primers (0.3 μM), 200 μl each deoxynucleoside triphosphate, 1× Expand High Fidelity buffer, 1.5 mM $MgCl_2$, and 2.6 U Expand High Fidelity PCR system enzyme mix. PCR consisted of the following: a denaturation step (94°C, 2 min); 10 cycles of denaturation (94°C, 15 s), annealing (50°C, 30 s), and extension (68°C, 8 min); 20 cycles of denaturation (94°C, 15 s), annealing (50°C, 30 s), and extension (68°C, 12 min); and a final elongation step (72°C, 7 min).

The primer sets used for amplifying the entire SCC*mec* region of strain JCSC290 and amplicon sizes are as follows: for the region from *orfX* to pT181 insert, cR1 and pT181-1 (6.3 kb); for the region from pT181 to *mecA*, pT181-2 and mA3 (7.2 kb); for the region from *mecA* to integrated ΨTn*554* (cad), mA4 and cad3 (8.8 kb); for the region flanking ΨTn*554* (cad), mN5 and mN23 (7.0 kb); for the region from ΨTn*554* (cad) to *ccr*, cad2, and β4 (7.2 kb); and for the region from *ccr* to chromosomal sequence flanking the left end of SCC*mec*, β3 and cLt3 (6.7 kb). DNA fragments encompassing the entire SCC*mec* region of JCSC1716 were amplified by long-range PCR with the same primer sets used for JCSC290 except for the following two sets: for the region from *orfX* to the IS*431* insert, cR1 and IS*431mec* (3.2 kb); for the region from *orfX* to the *mecA* gene, cR1 and mA3 (6.2 kb), mA4 and cad3 (8.7 kb), mN5 and mN23 (6. 8 kb), cad2 and β4 (7.4 kb), and β3 and cLt3 (6.7 kb). The locations of primers are indicated in Fig. 1. PCR products were purified with the QIA quick PCR purification kit (QIAGEN, Hilden, Germany) and sized by agarose gel electrophoresis.

PCR detection of spontaneous excision of the type 3A SCC*mec* and SCC*mercury* elements. Five primer sets were used to detect the precise excision of SCC*mec* and SCC*mercury* in strains 85/2082, JCSC290, and JCSC1716 by standard PCR using the same conditions as for SCC*mec* typing. The locations of primers are shown in Fig. 2, and their sequences are listed in Table 1. For strain 85/2082, primer pairs cR1 and cLt4, cR1 and mN19, and mN17 and cLt5 were used to detect the precise excision of type 3A SCC*mec*-SCC*mercury*, SCC*mercury*, and type 3A SCC*mec*, respectively. Primers cR1and cLt5 were used to detect *attB* (350 bp) in JCSC290 and JCSC1716.

Nested PCR was also carried out to detect the precise excision of SCC*mec* and SCC*mercury* following the procedure described previously (16).

Three sets of primer pairs were used: set A (the outer primers, cLt2 and cR2, and the inner primers, cLt5 and cR1); set B (the outer primers, cLt2 and mN20, and the inner primers, cLt5 and mN17); set C (the outer primers, cR2 and mN19, and the inner primers, mN18 and cR1). Set A was used to detect *attB* (350 bp) generated by the excision of type 3A SCC*mec* from strains JCSC290 and JCSC1716 or by the excision of a composite of type 3A SCC*mec* and SCC*mercury* from strain 85/2082. Set B and set C were used to detect the excision of type 3A SCC*mec* and SCC*mercury*, respectively, from 85/2082.

Multilocus sequence typing (MLST). MLST was performed as previously described (9). Alleles of the seven loci were assigned by comparing the sequences of each locus to those in the S. *aureus* MLST database (www.mlst.net). Sequence types were determined according to the pattern of combination of the seven alleles, and clonal complexes were defined by the BURST (based upon related sequence types) program available on the MLST website.

RESULTS AND DISCUSSION

A proposal for SCC*mec* element nomenclature. We propose a new nomenclatural system for SCC*mec* elements that has three features. The first feature is a description of SCC*mec* type. It appears to be widely accepted that SCC*mec* types are defined by *ccr* type and *mec* class. An SCC*mec* element can be simply described by *ccr* type (indicated by a number) and *mec* class (indicated by an uppercase letter). Alternatively, roman numeral designations could be used instead of combinations of *ccr* and *mec* as indicated in parentheses. These designations are as follows: type 1B (type I) for SCC*mec* elements carrying type 1 *ccr* and class B *mec*, type 2A (type II) for SCC*mec* elements carrying type 2 *ccr* and class A *mec*, type 3A (type III) for SCC*mec* elements carrying type 3 *ccr* and class A *mec*, type 2B (type IV) for SCC*mec* elements carrying type 2 *ccr* and class B *mec*, and type 5C (type V) for SCC*mec* elements carrying type 5 *ccr* and class C *mec*.

The second feature of our system is a description of J regions, which could be indicated after the SCC*mec* type

TABLE 1. List of primers used in this study

Primer function (detection)	Primer name (previous name)	Nucleotide sequence	Expected size of product	Location of primer in AB037671
Assignment for types of SCC*mec* elements				
ccr typing	βc (β2), α1 (α2), α2 (α3), α3 (α4)	See reference 14 or 29	Type 1, 0.7 kb; Type 2, 1 kb; Type 3, 1.6 kb	
Identification of *ccr* genes	αc, βc	See reference 29	Common: 0.56 kb	
ccrC	γR, γF	See reference 15	0.52 kb	
mec gene complex				
IS*431* L-*mecA*	IS2 (iS-2), mA2	See reference 29	2 kb	
IS*1272-mecA*	IS5, mA6	See reference 29	2 kb	
mecI-mecR1 (PB)	mI4, mcR5	See reference 29	0.64 kb	
mecA	mA1, mA2	See reference 29	0.28 kb	
Assignment for subtyping by the J-region difference in SCC*mec* elements				
2A1 (IIa)	2a1, 2a2	See reference 12 or 21	0.46 kb	
2A2 (IIb)	2b1, 2b2	See reference 12	0.85 kb	
2B1 (IVa)	4a1, 4a2	See reference 12 or 21	0.45 kb	
2B2 (IVb)	4b1, 4b2	See reference 12 or 21	1 kb	
2B3 (IVc)	4c1, 4c2	See reference 12 or 21	0.67 kb	
2B4 (IVd)	4d1, 4d2	See reference 12 or 21	1.0 kb	
MLEP (previously called MREP)	cR4	5′-GTTCAAGCCCAGAAGCGATGT-3′	Type i, 1.6 kb	
	mR5	5′-ATGCTCTTTGTTTTGCAGCA-3′	Type ii, 1.7 kb	
	mR6	5′-ATATTCTAGATCATCAATAGTTG-3′	Type iii, 1 kb	
ant(*4′*)-1 in SCC*mec*	mD1	5′-TAAGTGCTGACGCCTGAGGGAAT-3′	2.02 kb	
	am1	5′-CAGACCAATCAACATGGCACC-3′		
mer operon	merA2	5′-TCTTCACAGCCTGTGCATGTCATGCCT-3′	1.55 kb	
	merG	5′-TGATACCGCGAATGAATCAAAGGT-3′		
J region in SCC*mercury*	mN21	5′-TCATCTTTAACTACGATGGTGT-3′	0.58 kb	
	mN22	5′-ACTACAGCCATCTTCAGATAGA-3′		
Precise excision of SCC elements				
	cLt2	5′-ATCTTCTGAAGGATAATTCGCA-3′		212–233
	cR2	5′-AAACGACATGAAAATCACCAT-3′		
	cLt5	5′-TTCAATGTCGCTAAGTGCATTTT-3′		757–779
	cR1	5′-AAGAATTGAACCAACGCATGA-3′		67988–67968
	mN17	5′-TGTCGTTGTCTAAGTTTTCTAACT-3′		36057–36033
	mN18	5′-TATTGTAGTGTATATCACGATGT-3′		34705–34727
	mN19	5′-AGCACTATGGGCATTGATTGGA-3′		35414–35435
	mN20	5′-TGTTGCTTCCTGGGGATATCCATACT-3′		36948–36923
Amplification of type 3A SCC*mec* elements[a]				
	mA4	5′-TTACTGCCTAATTCGAGTGCT-3′		25830–25851
	pT181-1	5′-TAACTTCAGCATCTGCATTATCT-3′		30135–30157
	pT181-2	5′-AGGTTTATTGTCACTACAATTGA-3′		32869–32847
	mN23	5′-AGTAACGCAACGGGTATGATTA-3′		10641–10662
	cad2	5′-TAACTGCAACGGTCATCAGGGT-3′		14514–14493
	cad3	5′-TATCCTATTGATTGGGATAACCTT-3′		16925–16948
	mN5	5′-TTGCTTCGGGACTTACCTCTAGT-3′		17624–17646
	β-4	5′-AGATGTATACGCGGATAGAGGTA-3′		6934–6956
	β-3	5′-AGTTCCTTTACCGTGGACTTGA-3′		7675–7654
	cLt3	5′-TCCGCTACTTATGATACGCTTCTG-3′		877–900

[a] The sequences of primers cR1, mA3, and IS*431 mec* could be inferred from reference 22.

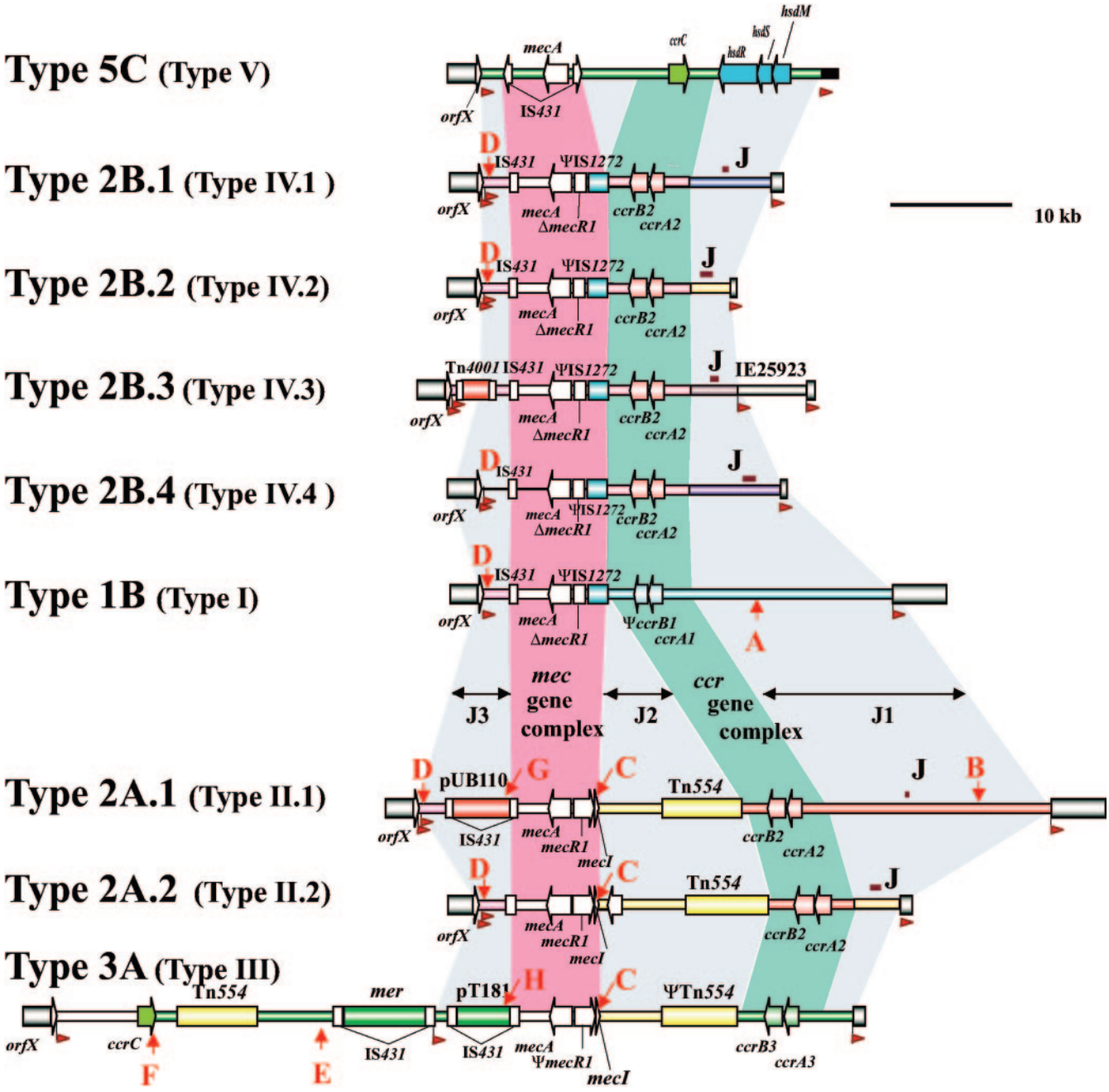

FIG. 1. Structural comparison of SCC*mec* elements. The structures of SCC*mec* elements are based on the nucleotide sequences deposited in the DDBJ/EMBL/GenBank databases under accession nos. AB033763 (type 1B SCC*mec*), D86934 (type 2A.1 SCC*mec*), AB127982 (type 2A.2) AB037671 (type 3A SCC*mec*), AB063172 (type 2B.1 SCC*mec*), AB063173 (type 2B.2 SCC*mec*), AB096217 (type 2B.3 SCC*mec*), and AB121219 (type 5C). The SCC*mec* element is composed of two essential gene complexes, the *ccr* gene complex (blue-green) and the *mec* gene complex (peach). The *ccr* gene complex consists of *ccr* genes that are responsible for the mobility of SCC*mec* and surrounding ORFs. The *mec* gene complex is responsible for methicillin-cephem resistance. Other areas (light gray) of SCC*mec* are nonessential and are divided into three regions, J1 to J3. Various drug resistance genes are found within the J2 and J3 regions of some SCC*mec* elements. Some ORFs specific for each type are found within the J1 region, such as *pls* in type I SCC*mec* and the *kdp* operon in type II SCC*mec*. Direct-repeat-containing integration site sequences of SCC*mec* elements are indicated by red arrowheads. The locations of primer sets (A to H) used for the multiplex PCR method developed by Oliveira and Lencastre (31) are indicated by red arrows. The locations of primer sets used for the identification of the J1 region (J) of type 2A and type 2B SCC*mec* element used in this study are indicated by red bars.

designation with numbers representing differences in the J1 region and J2-J3 regions, each separated by a period, e.g., 2A.1.1. The subtypes of type 2A and type 2B SCC*mec* elements would be described in numerals in the order that they are found, as follows (previous names are indicated in parentheses): type 2A.1 (type IIa), type 2A.2 (type IIb), type 2B.1 (type IVa), type 2B.2 (type IVb), type 2B.3 (type IVc), and type 2B.4 (type IVd). The structures of five SCC*mec* types and their

subtypes are shown in Fig. 1. They are indicated with the proposed new names.

The third feature of the system concerns enumeration. The *ccr* type or J-region identity could be numbered only in chronological order according to the time of identification. The objective of the new nomenclature is to resolve problems in naming of SCC*mec* elements. In 2001, Oliveira et al. reported the *ccrA4* and *ccrB4* genes, which shared weak similarity with three previously reported *ccr* genes found in a pediatric strain, HDE288 carrying class B *mec*, and designated the new SCC*mec* element type IV SCC*mec* (30). Independently, we found SCC*mec* elements carrying class B *mec* and type 2 *ccr* in C-MRSA strains isolated at the University of Chicago Children's Hospital and designated these type IV SCC*mec* (22). Since MRSA strains carrying the latter elements are found mostly in C-MRSA strains isolated in the United States or Europe, many researchers have regarded the elements carrying type 2 *ccr* and class B *mec* as type IV SCC*mec*. If the proposed nomenclature were adopted, the type IV SCC*mec* element found in HDE288 would be called type 4B. As an alternative to roman numerals, the element carried by HDE288 would be the prototypic type IV, since it was reported in 2001. However, at present, type IV SCC*mec* is regarded as an element that carries class B *mec* and type 2 *ccr*, and type V SCC*mec* is regarded as an element carrying class C *mec* and type 5 *ccr*. We suggest that it is reasonable to regard the element that is carried by HDE288 as a sixth *ccr* and *mec* combination, consisting of type 4 *ccr* and class B *mec*.

Similarly, if we are allowed to rename the SCC*mec* elements reported by other researchers (20, 23, 30, 36), we would like to do so as shown in Table 2. For example, type IA SCC*mec*, which carries plasmid pUB110 near *mecA* gene, could be renamed type 1B.1.2 (or type I.1.2). In this name, 1B (or I) signifies the combination of *ccr* type and *mec* class, "1" signifies that the J1 region carrying the *pls* gene is the same as that carried by strain NCTC10442, and "2" signifies that there is a difference concerning the presence of pUB110 in the J2 and J3 regions with respect to that carried by NCTC10442. As one of seven variants, SCC*mec* type IIB was reported by Shore et al. (36), which could be renamed 2A.3.2 (II.3.2). In this designation, 2A (or II) signifies the combination of *ccr* type and *mec* class, "3" signifies that the J1 region is not the same as that carried by previously determined type 2A SCC*mec* elements, and "2" signifies that this is the second type identified among type 2A.3 elements. Accordingly, the type IIB SCC*mec* element reported by Shore et al. and the type IIb SCC*mec* reported by Hisata et al. (12) can be distinguished easily by describing them as 2A.3.2 (II.3.2) and 2A.2 (II.2), respectively.

Identification of types of SCC*mec* elements distributed in Asian MRSA strains. All 615 strains tested by PCR were *mecA* positive, and their SCC*mec* types were assigned by PCR. The *ccr* types and *mec* classes were determined by the procedures and primer sets listed in Table 1. As shown in Table 3, 610 of 615 (99.2%) of MRSA strains could be classified with respect to four types of SCC*mec* elements: type 3A, 370 strains; type 2A, 207; type 2B, 32; type 1B, 1. Among five strains that could not be classified into any of the four types, three carried type 2 *ccr*, and two did not carry any *ccr* genes identified so far by PCR with three sets of primers listed in Table 1 for *ccr* typing, for identification of *ccr* genes, and for *ccrC*.

Type 3A SCC*mec* strains predominated in isolates from nine countries: Saudi Arabia, 18/19 strains (94.7%); India, 37/37 strains (100%); Sri Lanka, 29/29 strains (100%); Singapore, 87/87 strains (100%); Indonesia, 60/60 strains (100%); Thailand, 49/49 strains (100%); Vietnam, 34/36 strains (94.4%); Philippines, 14/15 strains (93.3%); and China, 40/40 strains (100%). This type was minor in Korean strains, 2/105 strains (2.0%), and it was not identified in Japanese strains. Type 2A SCC*mec* strains predominated in Japan and Korea, 126/138 strains (91.3%) and 79/105 strains (75.2%), respectively. The frequency of type 2B SCC*mec* strains was very low (5.3%), and they were identified in four countries: Saudi Arabia (1 strain), Philippines (1 strain), Korea (24 strains), and Japan (6 strains).

Subtyping of the J1 region and the *mec* left extremity. The type 2A and type 2B SCC*mec* elements could be further subtyped by differences in the J1 regions. The majority of type 2A SCC*mec* strains (189/207 strains [91.3%]) belonged to subtype 1 (formally designated subtype a). One hundred nineteen of 126 (94.4%) Japanese strains and 70 of 79 (88.6%) Korean strains carried the type 2A.1 SCC*mec* element. A classification of type 2A SCC*mec* element carried by the other nine Korean strains and seven Japanese strains could not be determined by PCR with primer sets specific for subtypes 1 and 2 (formally designated subtype b). In the case of the Korean strains, 12 of 24 were type 2B.1 SCC*mec* and 8 of 24 were type 2B.3 SCC*mec*, while all six Japanese strains were type 2B.3 SCC*mec*. The remaining six type 2B strains were unclassifiable when tested with the primer sets specific for four subtypes (subtypes 1, 2, 3, and 4) of type 2B SCC*mec* elements.

We have previously reported that nucleotides at the right extremities of three SCC*mec* elements (type I, type II, and type III) are characteristic, and we have developed a PCR method to distinguish SCC*mec* elements with a set of three primers that recognize the chromosomal region that is common to all *S. aureus* strains and the others recognizing regions specific for each type that are located at the right extremity of SCC*mec* elements. We had previously designated this method as *mec* right extremity polymorphism (MREP) typing (14). However, after the determination of the whole-genome sequences of strains N315 and Mu50, we realized that the SCC*mec* elements are located downstream of *orfX* on the chromosomal map. To be consistent with published genome maps, we have revised the illustration of the structure of SCC*mec* elements by placing *orfX* at the left as shown in Fig. 1. Hence, the region just downstream of *orfX*, which we initially called the *mec* right extremity, is now regarded as the left extremity of the SCC*mec* element. Accordingly, MREP is now referred to as *mec* left extremity polymorphism (MLEP) typing. In the present study, we performed MLEP testing for all 615 MRSA isolates and compared the left extremities to SCC*mec* types. We found that 203 of 207 (98.0%) of type 2A SCC*mec* strains possessed MLEP type ii, 320 of 370 (86.4%) type 3A SCC*mec* strains possessed MLEP type iii, and the sole type 1B SCC*mec* strain possessed MLEP type ii. These results demonstrate that there is a strong correlation between the SCC*mec* element type and MLEP type. Among type 2B SCC*mec* strains, most (28 of 32) carried MLEP type ii, while 3 carried MLEP type i, and 1 is still untypeable.

Among 50 (13.6%) type 3A SCC*mec* strains that showed discrepancies, 39 (10.5%) carried the type ii left extremity and the other 11 (3.0%) could not be classified as either type. Since this

TABLE 2. Uniform nomenclature for SCC*mec* elements

SCC*mec* type name		*ccr* type	*mec* class	Characteristic feature in:		Strain (sequence)	Reference(s)[a]
Reported	Proposed (alternative)			J1 region	J2 and J3 regions		
I	1B.1.1 (I.1.1)	1	B	*pls*		NCTC10442 (AB033763), COL (http://www.tigr.org)	14
IA	1B.1.2 (I.1.2)	1	B	*pls*	Carries pUB110	PER34	30
IIa	2A.1.1 (II.1.1)	2	A	*kdp* operon	Carries pUB110	N315 (D86934, http://www.bio.nite.go.jp /dogan/DOGAN_html /n315/general_feature.jsp), MRSA252 (http://www.sanger.ac.uk /Projects/S_aureus/)	16
II variant	2A.1.2 (II.1.2)	2	A	*kdp* operon	Does not carry pUB110		4
IIb	2A.2 (II.2)	2	A	Region specific for type IIb		JCSC3063 (AB127982)	12
IIA	2A.3.1 (II.3.1)	2	A	Same as that of IVb	Insertion of IS *1182*		36
IIB	2A.3.2 (II.3.2)	2	A	Same as that of IVb		(AJ810123)	36
IIC	2A.3.3 (II.3.3)	2	A	Same as that of IVb	Insertion/deletion of IS *1182*		36
IID	2A.3.4 (II.3.4)	2	A	Same as that of IVb	Insertion of IS *1182*, does not carry pUB110		36
IIE	2A.3.5 (II.3.5)	3	A	Same as that of IVb	Insertion/deletion of IS *1182*, does not carry pUB110	AR13.1/330.2 (AJ810120)	36
III	3A.1.1 (III.1)	3	A			85/2082 (AB037671), ANS46	14, 30
IIIA	3A.1.2 (III.1.2)	3	A		Does not carry pT181, does not carry *ips*	HU25 (AF422651-AF422696)	30
IIIB	3A.1.3 (III.1.3)	3	A		Does not carry pT181	HDG2	30
	3A.1.4 (III.1.4)	3	A		Carries MLEP type ii left extremity		This study
IVa	2B.1 (IV.1)	2	B	Specific for type IVa		CA05 (AB063172), MW2 (http://www.bio.nite.go.jp /dogan/DOGAN_html/mw2 /general_feature.jsp)	22
IVb	2B.2.1 (IV.2.1)	2	B	Specific for type IVb		8/6-3P (AB063173)	22
IVc	2B.3.1 (IV.3.1)	2	B	Specific for type IVc		81/108 (AB096217)	13
IVc	2B.3.2 (IV.3.2)	2	B	Specific for type IVc	Does not carry Tn*4001*	2314 (AY271717)	23
IVE	2B.3.3 (IV.3.3)	2	B	Same as that of IVc	Left extremity is different from that of IVc	AR43/3330.2 (AJ810121)	36
IVF	2B.2.2 (IV.2.2)	2	B	Same as that of IVb	Left extremity is different from that of IVb		36
IVd	2B.4 (IV.4)	2	B	J1 region specific for type IVd		JCSC4469 (AB097677)	12
IV	4B (VI.1)	4	B			HDE288 (AF411935)	30
IVA[b]	2B.N.2 (IV.N.2)	(2)	(B)		Carries pUB110	Not described	4
IVg	2B.5 (IV.5)	2	B			M03-68 (DQ106887)	20
V	5C.1 (V.1)	5	C			WIS (AB121219)	15

[a] The genome papers were not listed.
[b] Judging from the paper, the differences in J1 region, class of *mec*, and type of *ccr* were not examined. The data in parentheses for *ccr* type and *mec* class were given tentatively.

TABLE 3. Distribution of SCC*mec* elements in 11 Asian countries

SCC*mec* type	*ccr* gene complex	*mec* gene complex	Subtype	MLEP typing	*ccrC*	No. of strains tested from the following country:											No. of strains from 11 Asian countries	Total no. of strains (%)
						Saudi Arabia (n = 19)	India (n = 37)	Sri Lanka (n = 29)	Singapore (n = 87)	Indonesia (n = 60)	Thailand (n = 49)	Vietnam (n = 36)	Philippines (n = 15)	China (n = 40)	Korea (n = 105)	Japan (n = 138)		
3A(III)	3	A		iii	+	17	29	28	78	60	48	32	14	6	1		313	370 (60.2)
				iii	−			1	4			1			1		7	
				ii	−	1			2		1	1					5	
				ii	+									34			34	
				NT[a]	+		7		1								8	
				NT	−		1		2								3	
2A(II)	2	A	1(a)	ii	−										70	117	187	207 (33.67)
			1(a)	NT	−											2	2	
			n	ii	+										1		1	
			n	ii	−							2			8	5	15	
			n	NT	−											2	2	
2B(IV)	2	B	1(a)	ii	+										12		12	32 (5.20)
			1(a)	ii	−										3		3	
			3(c)	ii	−										5	3	8	
			3(c)	i	−											3	3	
			n	ii	+								1		2		3	
			n	ii	−										2		2	
			n	NT	−	1											1	
1B(I)	1	B		ii	−											1	1	1 (0.16)
2N	2	N		ii	−											3	3	5 (0.81)
NN	*ccr* (−)[b]	N		ii	−											2	2	

[a] NT, nontypeable.
[b] Negative in PCR experiment with a set of primers, αc and βc.

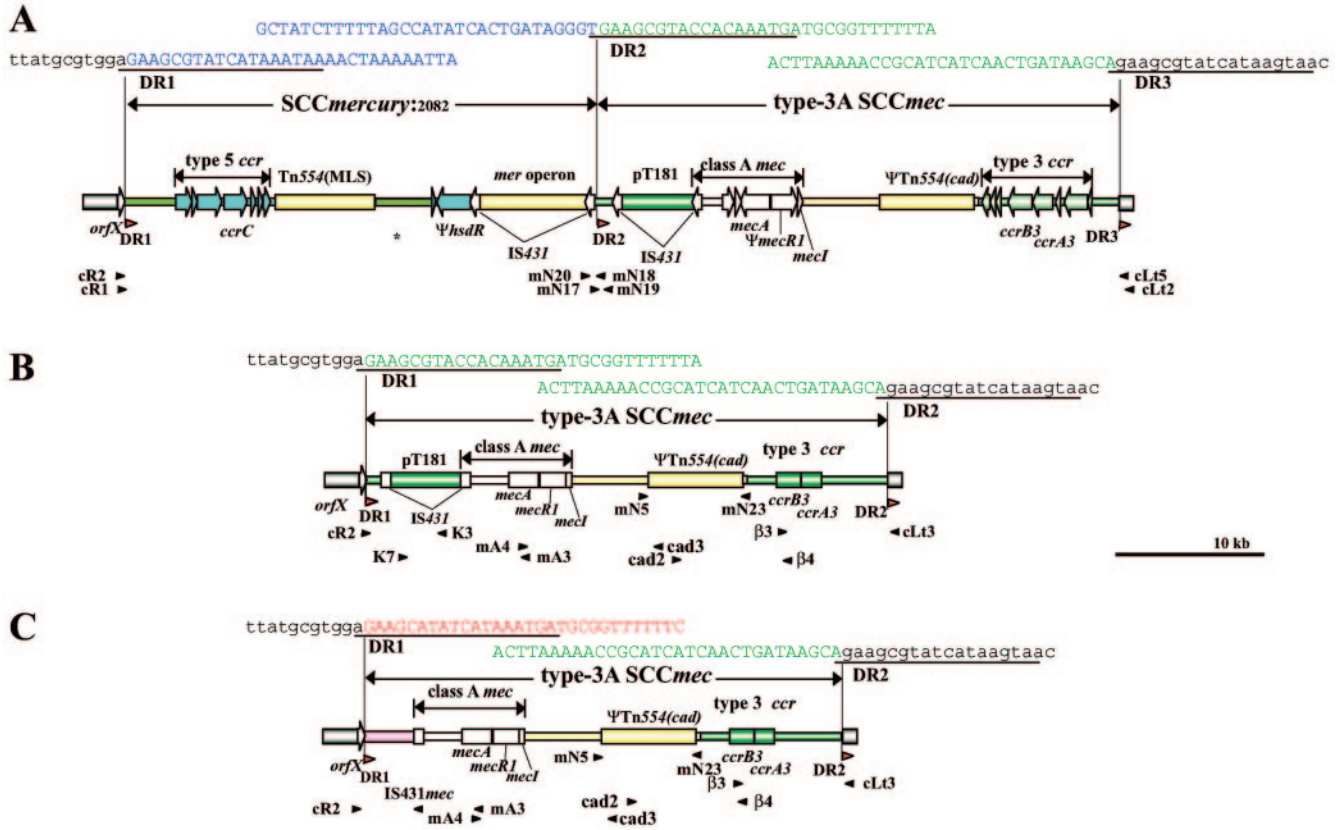

FIG. 2. Identification of small type 3A SCC*mec* elements. The structures of three type 3A SCC*mec* elements and characteristic nucleotides at both extremities are illustrated. (A) The structure of the type 3A SCC*mec* element and of the SCC*mercury* element (formerly called type III SCC*mec*) is based on the nucleotide sequences deposited in DDBJ/EMBL/GenBank databases under accession no. AB037671. (B) The structure of the type 3A SCC*mec* elements (3A.1.1) of strain JCSC290 is derived by PCR. (C) The structure of the type 3A SCC*mec* elements of strain JCSC1716 (3A.1.4) is derived by PCR. Black arrowheads indicate the locations of primers used for detecting the precise excision of SCC*mec* or SCC*mercury* and for amplification of the entire region containing the two type 3A SCC*mec* elements carried by JCSC290 and JCSC1716. The nucleotide sequences of the primers are listed in Table 1. The nucleotide sequences of both extremities of SCC*mercury* and SCC*mec* elements are illustrated. Red arrowheads indicate directly repeated sequences that constitute the integration site of SCC*mec* (ISS), and their nucleotide sequences are underlined.

was unexpected, we confirmed this result with a primer set that recognizes the *dcs* (downstream constant sequence) region or locus D, which has been previously tested by multiplex PCR (31, 32). All 39 strains were confirmed to be positive for the D locus.

A composite of SCC*mercury* and type 3A SCC*mec*. The *ccrC* gene encodes a site-specific recombinase that is responsible for the mobility of the type 5C (V) SCC*mec* element by mediating its precise excision and integration of the element into the chromosome (15). However, the localization of the *ccrC* gene is not restricted in type 5C SCC*mec* elements. It had been found as CZ072 in the type III SCC*mec* element of strain 85/2082 (accession no. AB037671) as reported previously (14). Soon after we reported the type III SCC*mec* element of 85/2082 as being the longest such element (67 kb), we noticed three integration site sequences (ISSs) located in and around the element (Fig. 2), which suggested that the long region could be a composite of two SCC elements, one carrying *ccrC* and the other carrying *ccrA3 ccrB3* as site-specific recombinases (11). In the present study, we carried out PCR to test this idea using validate the presumption using MRSA 85/2082 chromosomal DNA as a template and primers sets designed around ISSs to determine whether the two SCC elements independently excise. No PCR product was obtained in a standard PCR experiment with three primer sets that allow detection of the precise excision of two SCCs (cR1 and cLt4), type 3A SCC*mec* (mN17 and cLt5), and an SCC carrying *ccrC* (cR1 and mN19). Therefore, we then tried to identify precise excision by a nested PCR approach and found that DNA fragments containing *attB* could be amplified, although the frequency of excision of either element alone was lower than that of both elements concomitantly.

These data show that an SCC element carrying the mercury resistance operon and the site-specific recombinase *ccrC* can be distinguished from type 3A SCC*mec* elements carrying class A *mec* and type 3 *ccr*. Therefore, we have designated this element SCC*mercury*.

To determine the distribution of SCC*mercury*, we conducted a PCR experiment to identify the *ccrC* gene, the mercury resistance determinant, and the junkyard region. The results showed that 371 strains carried the *ccrC* gene. Among these, type 3A SCC*mec* was found in 355 strains, of which 313 carried MLEP type iii (Table 3). Since 310 of 313 strains were positive for PCR to detect

the mercury resistance determinant and/or junkyard region, they can be considered to possess an SCC*mercury* element that is similar to that of strain 85/2082. Other *ccrC*-positive type 3A SCC*mec* strains included 34 Chinese strains carrying MLEP type ii, seven Indian strains, and a Singapore strain whose MLEP was untypeable. Interestingly, a Korean type 2A SCC*mec* strain and 15 Korean type 2B strains carried *ccrC*. Our PCR analyses have not identified the *ccrC* gene in the type 2A or type 2B SCC*mec* element. These strains may carry elements encoding *ccrC* integrated at a position different from that of *orfX* (Y. Kondo, unpublished data).

Mercuric chloride was an antiseptic used in the past, and *S. aureus* originally became resistant to this agent by the acquisition of plasmids encoding mercury resistance determinants, such as pI258 (24, 27, 35). Nowadays, mercuric chloride is no longer widely used in hospitals, and the mechanism of acquisition of SCC*mercury* by *S. aureus* has not been clarified. One possibility is that a coagulase-negative staphylococci (C-NS) acquired a mercury resistance determinant that permitted survival under the pressure of mercuric chloride, and this determinant may have integrated into an SCC element to form SCC*mercury* in C-NS, from which it was transferred to *S. aureus*. Alternatively, a plasmid-encoded determinant may have been transferred to *S. aureus* and it then integrated into an SCC element to form SCC*mercury*. SCC*mercury* may be a good example of an SCC element that can acquire exogenous genes to promote the survival of staphylococci under selective pressures. It is plausible that MSSA strains carrying an element encoding *ccrC* or similar to SCC*mercury* were preexisting and were changed to MRSA strain by the integration of an SCC*mec* element. Dominant strains carrying both type 3A SCC*mec* and SCC*mercury*, as represented by strain 85/2082, may have arisen by the integration of type 3A SCC*mec* into MSSA strains carrying SCC*mercury*.

Identification of two small type 3A SCC*mec* elements. We speculated that if the type III SCC*mec* element of strain 85/2082 is a composite of two SCC elements, there may be strains that carry only the type 3A SCC*mec* element. To identify such strains, we conducted PCR experiments and identified two small type 3A SCC*mec* elements whose junkyard regions downstream of *orfX* were different (Fig. 2). One was found by screening 11 MLEP untypeable strains by PCR with the primer set cR2 (specific for *orfX*) and mN19 (located downstream of DR2). A DNA fragment of 1 kb was successfully amplified only from the Singaporean isolate JCSC290. Subsequently, the entire region of the type 3A SCC*mec* element in JCSC290 was amplified by long-range PCR with primer sets specific for essential constituents of the type 3A SCC*mec* elements of 85/2082. The size of the type 3A SCC*mec* element carried by JCSC290 was almost identical to that of 85/2028 as judged by agarose gel electrophoresis. The nucleotide sequences of both ends of the JCSC290 type 3A SCC*mec* element were identical to the corresponding ends of the type 3A SCC*mec* element of 85/2082 (Fig. 2).

The other element was identified in the Saudi Arabian isolate JCSC1716, for which we had tried to determine the structure of SCC*mec* element that lacked *ccrC* but carried MLEP type ii. The entire region of the type 3A SCC*mec* element carried by JCSC1716 was approximately 31 kb in size, as estimated by long-range PCR. Two direct repeats and incomplete inverted repeats were also identified at the ends of the element (Fig. 2).

We also carried out PCR to investigate whether the type 3A SCC*mec* elements precisely excise from the chromosome. When chromosomal DNAs of strains JCSC290 and JCSC1716 were used as templates, a 0.35-kb DNA fragment containing *attBscc* was successfully amplified, indicating that small type 3A SCC*mec* elements can be excised.

According to the new nomenclature, the SCC*mec* element carried by strain JCSC290 is called type 3A.1.1, and the element carried by strain JCSC1716 is called type 3A.1.4. Oliveira et al. reported two type III SCC*mec* variants, IIIA and IIIB (30). Since type IIIA does not carry plasmid pT181 in the J3 region, it may be regarded as type 3A.1.2. As for type IIIB, it closely resembles the SCC*mec* element carried by JCSC290 except that it does not carry plasmid pT181. Since this element was reported in 2001, it may be regarded as type 3A.1.3., indicating that it is the third variant found with differences in the J2 and J3 regions of type 3A.1 SCC*mec*. The IIIA and IIIB types would differ in the presence of *ips* (the region between the IS*431* right flanking copy of pT181 and the IS*431* left flanking copy of pI258), if the SCC*mercury* region is omitted from the illustrated structure of type IIIA SCC*mec*. Furthermore, it is noteworthy that the type 3A SCC*mec* elements are not large and that previously reported type III SCC*mec* strains have a long region composed of two SCC*mec* elements.

Characteristics and evolution of Asian MRSA clones. The genotypes of representative isolates were determined by multilocus sequence typing. As shown in Table 4, three strains that carry dominant SCC*mec* types and as many strains that carry minor SCC*mec* types in 11 countries were chosen and their genotypes were determined by MLST. MLST analysis revealed that the dominant MRSA strains in Korea and Japan belonged to clonal complex 1 (CC1) or CC5, whereas the dominant MRSA strains of the other nine countries belonged to CC8 (Table 4). CC5 type 2A SCC*mec* clones predominate in Korea and Japan, and CC8 type 3A SCC*mec* clones predominate in the other nine countries as reported by Ko et al. (19).

When we investigated SCC*mec* types in detail, we noticed that the MRSA clones distributed in Asian countries were surprisingly diverse and that they exhibited characteristic features in each country. We tested whether CC5 type 2A SCC*mec* strains carry the *ant*(*4′*)-1 gene near the *mecA* gene and found that the frequency of this gene differed among strains isolated from two countries. Among 126 Japanese type 2A.1 strains, 121 (96%) carried the *ant*(*4′*)-1 gene downstream of *mecA*, whereas only 42 of 79 (53%) Korean type 2A.1 SCC*mec* strains carried it. These data agree well with that of Cha et al., who reported that 32 of 60 (53.3%) type 2A.1 SCC*mec* strains did not carry plasmid pUB110, and they called this element a type II variant (4). The type II SCC*mec* element that does not carry plasmid pUB110 can be renamed type 2A.1.2 (II.1.2) according to our proposed nomenclature.

Among the minority clones in each country, most were type 3A SCC*mec* strains, other than a Singapore isolate belonging to CC8. Other minor clones carrying type 2A or type 2B SCC*mec* elements belonged to CC1, CC5, CC8, or CC36.

The MLST genotypes of Korean and Japanese MRSA strains carrying SCC*mec* elements other than type 2A.1 did not always differ from those of major clones, suggesting that these

TABLE 4. Genotypes of representative MRSA strains assigned by MLST

Country	Strain	Allele profile							ST	CC	SCC*mee-ccrC*-MLEP type
		arc	*aro*	*glp*	*gmk*	*pta*	*tpi*	*yqiI*			
Saudi Arabia	JCSC1705	2	3	1	1	4	4	3	239	8	3A-*ccrC*-iii
	JCSC1715	2	3	1	1	4	4	3	239	8	3A-*ccrC*-iii
	JCSC1719	2	3	1	1	4	4	3	239	8	3A-*ccrC*-iii
	JCSC1716	2	3	1	1	4	4	3	239	8	3A-ii
	JCSC1718	1	4	1	4	12	1	10	5	5	2B-NT[a]
India	JCSC221	2	3	1	1	4	4	3	239	8	3A-*ccrC*-iii
	JCSC240	2	3	1	1	4	4	30	241	8	3A-*ccrC*-iii
	JCSC245	2	3	1	1	4	4	3	239	8	3A-*ccrC*-iii
	JCSC234	2	3	1	1	4	4	3	239	8	3A-*ccrC*-NT
	JCSC235	2	3	1	1	4	4	3	239	8	3A-NT
Sri Lanka	JCSC1590	2	3	1	1	4	4	3	239	8	3A-*ccrC*-iii
	JCSC1591	2	3	1	1	4	4	3	239	8	3A-*ccrC*-iii
	JCSC1592	2	3	1	1	4	4	3	239	8	3A-*ccrC*-iii
Singapore	JCSC250	2	3	1	1	4	4	3	239	8	3A-*ccrC*-iii
	JCSC282	2	3	1	1	4	4	3	239	8	3A-*ccrC*-iii
	JCSC289	2	3	1	1	4	4	3	239	8	3A-*ccrC*-iii
	JCSC235	2	3	1	1	4	4	3	239	8	3A-NT
	JCSC284	2	3	91	1	4	4	3	614	8	3A-ii
	JCSC298	2	3	1	1	4	4	3	239	8	3A-iii
	JCSC294	1	1	1	1	12	4	1	363	1	3A-*ccrC*-NT
Indonesia	JCSC209	2	3	1	1	4	4	3	239	8	3A-*ccrC*-iii
	JCSC1608	2	3	1	1	4	4	3	239	8	3A-*ccrC*-iii
	JCSC1626	2	3	1	1	4	4	3	239	8	3A-*ccrC*-iii
Thailand	JCSC170	2	3	1	1	4	4	3	239	8	3A-*ccrC*-iii
	JCSC195	2	3	1	1	4	4	3	239	8	3A-*ccrC*-iii
	JCSC1568	2	3	1	1	4	4	3	239	8	3A-*ccrC*-iii
	JCSC1570	2	3	1	1	4	4	3	239	8	3A-ii
Vietnam	JCSC1659	2	3	1	1	4	4	30	241	8	3A-*ccrC*-iii
	JCSC1683	2	3	1	1	4	4	3	239	8	3A-*ccrC*-iii
	JCSC1685	2	3	1	1	4	4	3	239	8	3A-*ccrC*-iii
	JCSC1675	2	3	1	1	4	4	3	239	8	3A-ii
	JCSC1657	2	2	52	2	3	3	2	346	36	2A-ii
Philippines	JCSC1690	2	3	1	1	4	4	30	241	8	3A-*ccrC*-iii
	JCSC1697	2	3	1	1	4	4	30	241	8	3A-*ccrC*-iii
	JCSC1699	2	3	1	1	4	1	30	241	8	3A-*ccrC*-iii
	JCSC1692	3	32	1	1	4	4	3	254	8	2B-*ccrC*-ii
China	JCSC1536	2	3	1	1	4	4	3	239	8	3A-*ccrC*-ii
	JCSC1546	2	3	1	1	4	4	3	239	8	3A-*ccrC*-ii
	JCSC1555	2	3	1	1	4	4	3	239	8	3A-*ccrC*-ii
	JCSC199	2	3	1	1	4	4	3	239	8	3A-*ccrC*-iii
Korea	JCSC108	1	4	1	4	12	1	10	5	5	2A.1-ii
	JCSC117	1	4	1	4	12	1	10	5	5	2A.1-ii
	JCSC138	1	4	1	4	12	1	10	5	5	2A.1-ii
	JCSC120	1	4	1	4	12	1	10	5	5	2A.n-ii
	JCSC128	3	32	1	1	4	4	3	254	8	2A.n-*ccrC*-ii
	JCSC1475	50	3	1	1	4	4	3	344	8	3A-*ccrC*-iii
	JCSC1489	3	32	1	1	4	4	3	254	8	2B.1-ii
	JCSC1483	3	32	1	1	4	4	3	254	8	2B.1-*ccrC*-ii
	JCSC1477	3	32	1	1	4	4	63	345	8	2B.3-*ccrC*-ii
	JCSC1513	3	32	1	1	4	4	3	254	8	2B-*ccrC*-ii
	JCSC1495	1	1	1	1	1	1	1	1	1	2B.3-ii
	JCSC134	1	1	1	1	1	1	1	1	1	2B-ii
Japan	JCSC343	1	4	1	4	12	1	10	5	5	2A.1-ii
	JCSC486	1	4	1	4	12	1	10	5	5	2A.1-ii
	JCSC686	1	4	1	4	12	1	10	5	5	2A.1-ii
	JCSC590	1	4	1	4	12	1	10	5	5	2A.n-NT
	JCSC632	1	4	1	4	12	1	10	5	5	2A.n-ii
	JCSC709	1	1	1	9	1	1	1	81	1	2B.3-i
	JCSC454	1	1	1	9	1	1	1	81	1	2B.3-ii
	JCSC419	1	4	1	4	12	1	10	5	5	2C-i
	JCSC539	3	4	1	4	12	1	35	366	5	1B-ii
	JCSC581	1	4	1	4	12	1	10	5	5	2N-ii

[a] NT, nontypeable.

MRSA clones might have been generated by the integration of an SCC*mec* element that was distributed in each country as a major MSSA clone. When we closely examined CC8 type 3A SCC*mec* clones, we noticed that each was composed of a dominant clone ST239 carrying a composite of type 3A SCC*mec* and SCC*mercury* elements and a minor clone ST239 carrying type 3A SCC*mec*, whose junkyard region was not always conserved, and different SCC elements represented by strains JCSC1716, JCSC298, JCSC234, JCSC235, JCSC1570, JCSC1675, and the Chinese dominant clone. It is possible that both SCC*mercury* and type 3A SCC*mec* elements had produced many variants and that these variants had integrated into MRSA strains or had been disseminated in each country. Strains carrying a type 3A SCC*mec* element alone, such as JCSC290 and JCSC1716, may have been generated either by the integration of a type 3A SCC*mec* element into MSSA strains prevalent in each country or by the excision of SCC*mercury*.

As for Korean CC8 strains, a strain belonging to ST344 carried type 3A SCC*mec* element. These data quite agree well with those of Cha et al., who showed that Korean type III SCC*mec* strains belonged to ST239 or ST344 (4). In contrast, other strains carrying minor SCC*mec* elements such as 2A.n, 3A, 2B.1, 2B.3, and 2B.n belonged to ST254 and ST345. Interestingly, most of these type 2A SCC*mec* and type 2B SCC*mec* strains belonging to ST254 carried the *ccrC* gene. The reason why so many different SCC*mec* elements are integrated into ST254 strains remains unclear. Since ST254 was identified only in Korean strains in this study, we presume that Korean ST254 strains acquired the SCC*mec* element and were locally changed to MRSA strains.

It has been hypothesized that a certain SCC*mec* element was integrated into MSSA cells to generate a MRSA clone and that the SCC*mec* element was excised to generate MSSA, and another SCC*mec* element was integrated in it to form another MRSA clone (8). This might be one hypothesis for the evolution of MRSA. However, it was reported that methicillin-resistant determinants create large deletions upon excision (34, 38). As far as we can tell, the spontaneous precise excision of the SCC*mec* element from the MRSA strain is infrequent, and the frequency of the precise excision of *mecA* from IS*431* is approximately 1/10th that of the deletion frequency (Y. Katayama, N. Aritaka, et al. unpublished data). Another possibility is that SCC*mec* carried by methicillin-resistant C-NS might be integrated into MSSA to generate MRSA clones, but this does not directly conflict with the above hypothesis.

Concerning the distribution of major clones, the data agree very well with the previous reports that type 2A SCC*mec* strains are dominant in Korea and Japan and that type 3A SCC*mec* strains are dominant in the other nine countries (4, 8, 14, 19, 30). What is the reason for these geographical differences among MRSA clones? The explanation may lie in the fact that staphylococci inhabit the skin and mucous membranes of healthy humans and may be transported to other countries that are culturally or economically related. The data may suggest that these relations may influence the distribution of staphylococci as well as MRSA strains, since the CC5 type 2A1 SCC*mec* clone has been found in Korea, Japan, and the United States and the CC8 type 3A SCC*mec* clone is distributed in Asian countries where the influence of European countries is considerable. A worldwide analysis of MRSA clones and the study of the mechanism of acquisition of SCC elements from other species should help resolve these questions in the near future.

SCC*mec* typing in the past and in the future. Oliveira and Lencastre developed a multiplex PCR system to assign four types of SCC*mec* elements by identifying characteristic genes or motifs in junkyard regions (31). However, the use of the multiplex PCR for assignment of SCC*mec* elements raises some concerns. First, the presence of locus D (*dcs* region) is not confined to type 1B (type I), type 2A (type II), and type 2B (type IV) SCC*mec* elements. We showed in this study that locus D can be identified in 39 strains carrying type 3A SCC*mec* element. Second, the identification of type III SCC*mec* is problematic. We have reported that the type III SCC*mec* element carried by MRSA 85/2082 is the longest SCC*mec* element. Oliveira and Lencastre might agree with our concept and the element they have used as type III SCC*mec* turns out to be a composite of type 3A SCC*mec* and SCC*mercury*. Therefore, it could be said that identification of two loci that are used to assign type III SCC*mec* element, locus E (the region between the mercury operon and Tn*554*) and locus F (*ccrC*) shown in Fig. 1, signifies the carriage of SCC*mercury*. Third, types of SCC*mec* elements would not be determined by assigning J regions. There are many type 2A SCC*mec* elements which do not carry the *kdp* operon in the junkyard region and these would not be classified as type II by the multiplex PCR system. Another multiplex strategy for SCC*mec* assignment has been reported by Zhang et al. (40) and further developed by our group (Y. Kondo, T. Ito, X. X. Ma, S. Watanabe, B. N. Kreiswerth, J. Etienne, and K. Hiramatsu, unpublished data). Both systems share the strategy to identify *mec*, *ccr*, and the J1 region, although they differ slightly with respect to primer sequences and target genes. We expect that SCC*mec* elements could be assigned by first determining the *mec* class and *ccr* type and then by identifying differences in J regions with the sets of primers reported previously (12, 31, 40) or described in this paper.

With the increasing interest in MRSA, it is likely that numerous SCC*mec* elements will be found in the near future. Since establishing a new nomenclature requires the consensus of many dedicated staphylococcal researchers, a meeting is needed to solve the problematic issue of naming SCC*mec* elements. We proposed the usage of roman numerals as an alternative because they are simple, well-known, and already accepted. We have attempted to establish a basis for the nomenclature of SCC*mec* elements, and we invite the suggestions of the staphylococcal research community for improvements in the proposed nomenclature.

ACKNOWLEDGMENTS

We thank Mark C. Enright for his kind help with MLST.

This work was supported by a Grant-in-Aid for 21st Century COE Research and a Grant-in-Aid for Scientific Research on Priority Areas (13226114) from The Ministry of Education, Science, Sports, Culture and Technology of Japan.

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