Classification of Staphylococcal Cassette Chromosome mec (SCCmec): Guidelines for Reporting Novel SCCmec Elements*†

International Working Group on the Classification of Staphylococcal Cassette Chromosome Elements (IWG-SCC)*

Staphylococci, which frequently colonize the skin and mucous membranes of humans and animals, have the capacity to acquire antimicrobial resistance determinants rapidly, particularly after the introduction of new antimicrobial agents into clinical practice. Because *Staphylococcus aureus* shows the highest pathogenic potential among the many species of staphylococci, the acquisition of resistance determinants by *S. aureus* has presented the greatest challenge to the treatment and control of staphylococcal infections. Methicillin-resistant *S. aureus* (MRSA) strains are particularly important because they are a leading cause of health-care-associated infections worldwide, and have also emerged as a major cause of community-associated infections.

The defining feature of MRSA is the staphylococcal cassette chromosome mec (SCCmec). A detailed explanation for the term “SCCmec” is available at the website http://www.staphylococcus.net. This is a mobile genetic element that carries the central determinant for broad-spectrum beta-lactam resistance encoded by the mecA gene. The emergence of methicillin-resistant staphylococcal lineages is due to the acquisition and insertion of the SCCmec element into the chromosome of susceptible strains.

SCCmec elements are highly diverse in their structural organization and genetic content and have been classified into types and subtypes. It is now common practice to define MRSA clones by the combination of SCCmec type and the chromosomal background (i.e., sequence type [ST]), as defined by multilocus sequence typing) in which SCCmec resides (e.g., ST22-SCCmec IV, abbreviated as ST22-IV) (5). Many types, subtypes, and variants of SCCmec elements and SCC elements lacking mecA have been reported without following any standardized, internationally agreed rules of nomenclature. Consequently, there are ambiguities and inconsistencies in the classification of SCC elements in the published literature to date. To address these issues, the International Working Group on the Classification of Staphylococcal Cassette Chromosome Elements (IWG-SCC) was organized to (i) form an intellectual network to contribute to the study of SCC elements, (ii) establish a consensus on a uniform nomenclature system for SCC elements, (iii) define minimum requirements for the description of new SCC elements, and (iv) establish guidelines for the identification of SCC elements for epidemiological study (i.e., SCCmec typing). Herein, we propose guidelines for the classification of SCCmec and other SCC elements.

CHARACTERISTICS OF SCCmec ELEMENTS

Beta-lactams, which were among the first antimicrobial agents to be introduced into clinical practice, are still one of the most effective classes of antimicrobials used in human medicine. Methicillin, which was introduced in 1960, is a semisynthetic penicillin specifically designed for the treatment of infections caused by beta-lactamase-producing staphylococci. However, within 1 year following the introduction of methicillin into clinical practice, the first MRSA strains were reported from clinical infections. MRSA strains produce an additional penicillin-binding protein, known as either PBP2a or PBP2’,** which has a low affinity for most of the semisynthetic penicillins, e.g., methicillin, nafcillin, and oxacillin, as well as most cephin agents. PBP2a or PBP2’ is encoded by an acquired gene, mecA, which has been cloned and sequenced along with the genes that control its expression, mecR1 (encoding the signal transducer protein MecR1) and mecI (encoding the repressor protein MecI). When it became apparent that mecA was widely disseminated among multiple staphylococcal species, it was hypothesized that it could be carried on a mobile genetic element that could be easily transferred among staphylococcal species.

A genetic element that encoded methicillin resistance and carried unique site-specific recombinases designated as cassette chromosome recombinases (ccr) was subsequently identified and designated as SCCmec (8, 11). Soon after the initial description of SCCmec, several structurally different SCCmec elements were described. These elements shared several characteristics, as follows: (i) carriage of mecA in a mec gene complex, (ii) carriage of a ccr gene(s) (ccrAB and/or ccrC) in a ccr gene complex, (iii) integration at a specific site in the staphylococcal chromosome, referred to as the integration site sequence (ISS) for SCC, which serves as a target for ccc-mediated recombination, and (iv) the presence of flanking direct repeat (DR) sequences containing the ISS.
The basic concepts for the classification of SCCmec elements

SCCmec elements are classified by a hierarchical system into types and subtypes. Types are defined by the combination of (i) the type of ccr gene complex, which is represented by the ccr gene allotype, and (ii) the class of the gene complex. These are the key elements of the cassette responsible for integration and excision of SCCmec and the beta-lactam resistance phenotype, respectively (Table 1 and Fig. 1).

**ccr gene complex.** The ccr gene complex is composed of the ccr gene(s) and surrounding open reading frames (ORFs), several of which have unknown functions. Currently, three phylogenetically distinct ccr genes, ccrA, ccrB, and ccrC, have been identified in S. aureus with DNA sequence similarities below 50% (Fig. 2 and 3). The ccrA and ccrB genes that have been identified to date have been classified into four allotypes. In general, ccr genes with nucleotide identities more than 85% are assigned to the same allotype, whereas ccr genes that belong to different allotypes show nucleotide identities between 60% and 82%. All ccr variants identified to date have shown ≥87% similarity; thus, there is only one ccrC allotype. We suggest describing their differences as alleles by using previously used numbers, e.g., ccrCI allele 2 or ccrC1 allele 8.

The classification of ccr genes is summarized in Fig. 2. In the proposed nomenclature, novel ccr genes should be defined based on DNA sequence similarities of <50%, and novel allotypes of ccr genes should be designated if their DNA sequence similarity identities are between 50% and 85%. This convention should be followed for naming novel ccr genes. In the future, it may be necessary to define additional allotypes, including those of ccrC.

The phylogenetic relationships and DNA sequence similarities of representative ccr genes, including those identified in staphylococci other than S. aureus, are illustrated (Fig. 3 and see Table S1 in the supplemental material, respectively). Although sequences derived from staphylococci other than S. aureus frequently diverge from those identified in S. aureus isolates, all of them have been classified as ccrA, ccrB, or ccrC. In staphylococci other than S. aureus, a few extra ccr allotypes have been identified, as follows: ccrA5 is the ccrA gene of Staphylococcus pseudintermedius KM241, ccrB6 is the ccrB gene of Staphylococcus saprophyticus ATCC 15305, and ccrB7 is the ccrB gene of S. saprophyticus TSU33.

The ccr gene complexes should be numbered according to the timing of their description. To date, two distinct groups have been reported, one carrying two adjacent ccr genes, ccrA and ccrB, and the other carrying ccrC. Based on allelic variations in ccr, a series of allotypes has been defined. The ccr gene complex identified in S. aureus includes type 1 (carrying ccrAIB1), type 2 (carrying ccrA2B2), type 3 (carrying ccrA3B3), type 4 (carrying ccrA4B4), and type 5 (carrying ccrC), which can be detected by conventional PCR analysis with pairs of specific primers.

**mec gene complex.** The mec gene complex is composed of mecA, its regulatory genes, and associated insertion sequences. The class A mec gene complex (class A mec) is the prototype complex, which contains mecA, the complete mecRI and mecI regulatory genes upstream of mecA, and the hypervariable region (HVR) and insertion sequence IS431 downstream of mecA. The class B mec gene complex is composed of mecA, a truncated mecRI resulting from the insertion of IS272 upstream of mecA, and HVR and IS431 downstream of mecA. The class C mec gene complex contains mecA and truncated mecRI by the insertion of IS431 upstream of mecA and HVR and IS431 downstream of mecA. There are two distinct class C mec gene complexes; in the class C1 mec gene complex, the IS431 upstream of mecA has the same orientation as the IS431 downstream of mecA (next to HVR), while in the class C2 mec gene complex, the orientation of IS431 upstream of mecA is reversed. C1 and C2 are regarded as different mec gene complexes since they have likely evolved independently. The class D mec gene complex is composed of mecA and ΔmecRI but does not carry an insertion sequence downstream of ΔmecRI (as determined by PCR analysis) (12).

Several variants within the major classes of the mec gene complex have been described, including insertions of IS431 or ISI182 upstream of mecA in the class A mec gene complex or insertion of Tn4001 upstream of mecA in the class B mec complex. These variants are indicated by a numerical string following the class (e.g., class B3, indicated in Fig. 1).

**J regions: regions other than mec and ccr gene complexes.** Besides the mec and ccr gene complexes, the SCCmec element also contains three so-called J regions, which constitute non-essential components of the cassette. These regions may carry additional antimicrobial resistance determinants. They were first designated as the L-C, C-M, and I-R regions but were later changed to J regions. We propose that the term J region refers to “joining region,” rather than the previously used “junkyard region.”

**FIG. 1.** Basic structures of representative SCCmec elements. The structures of SCCmec elements of representative strains are illustrated based on the following nucleotide sequences deposited in databases: NCTC10442 (AB033763), N315 (D86934), 85/2082 (AB037671), CA05 (AB063172), ZH47 (AM292304), WIS (AB121219), TSGH17 (AB512767), PM1 (AB462393), HDE288 (AF411935), JCSC6082 (AB373032), and C10684 (FJ390057). Red arrowheads indicate the ISS of SCC that comprise DR sequences.

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**TABLE 1. SCCmec types identified in S. aureus**

<table>
<thead>
<tr>
<th>SCCmec type</th>
<th>ccr gene complex</th>
<th>mec gene complex</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>1 (A1B1)</td>
<td>B</td>
</tr>
<tr>
<td>II</td>
<td>2 (A2B2)</td>
<td>A</td>
</tr>
<tr>
<td>III</td>
<td>3 (A3B3)</td>
<td>A</td>
</tr>
<tr>
<td>IV</td>
<td>2 (A2B2)</td>
<td>B</td>
</tr>
<tr>
<td>V</td>
<td>5 (C)</td>
<td>C2</td>
</tr>
<tr>
<td>VI</td>
<td>4 (A4B4)</td>
<td>B</td>
</tr>
<tr>
<td>VII</td>
<td>5 (C)</td>
<td>C1</td>
</tr>
<tr>
<td>VIII</td>
<td>4 (A4B4)</td>
<td>A</td>
</tr>
</tbody>
</table>

a ccr genes in the gene complex are indicated in parentheses.
b ccrA4B4 genes found in type VIII SCCmec were nearly identical to those in the S. epidermidis SCC-CI element and showed nucleotide identities of 89.6% and 94.5% to those found in type V1 SCCmec.
Type I(1B) (NCTC10442)

Type II(2A) (N315)

Type III(3A) (85/2082)

Type IV(2B) (CA05)

Type IV(2B&5) (ZH47)

Type V(5C2) (WIS)

Type V(5C2&5) (TSGH17, PM1)

Type VI(4B) (HDE288)

Type VII(5C1) (JCSC6082)

Type VIII(4A) (C10682)
J1 (formerly L-C) is the region between the right chromosomal junction and the ccr complex, J2 (C-M) is between the ccr gene complex and the mec gene complex, and J3 (I-R) is between the mec complex and the left chromosomal junction. Variations in the J regions within the same mec-ccr complex are used for defining SCC mec subtypes.

EIGHT CURRENTLY ESTABLISHED SCC mec TYPES

To date, eight SCC mec types have been described for S. aureus using the criteria described above (Table 1 and Fig. 1). The first three SCC mec elements were designated as types I, II, and III (8, 9). These were followed by reports of SCC mec types IV to VIII (1, 10, 15, 19, 23). This nomenclature should be retained, but an additional (more informative) system for naming the novel SCC mec elements, based on the type of ccr and class of mec present, is proposed. For example, type I (1B) SCC mec indicates an SCC mec harboring a type 1 ccr and a class B mec gene complex. The other known SCC mec types would be designated type II (2A), type III (3A), type IV (2B), type V (5C2), type VI (4B), type VII (5C1), and type VIII (4A). Thus, SCC mec types should be designated by roman numerals in the order in which they are reported, followed by the ccr gene complex and the mec gene complex together in parentheses.

Type III and VI SCC mec elements have been revised based on additional sequence information. The type III SCC mec element was reported to be 67 kb in length and was considered to be the longest SCC mec element (9). However, in 2006, it was reported that this 67-kb element was a composite of two smaller SCC elements; SCC mercury and type III (3A) SCC mec (carrying type 3 ccr and class A mec) integrated in tandem (4). To avoid confusing SCC mercury with SCC mec, the name of the former is changed to SCCHg. The SCC mec element carried by S. aureus strain HDE288 was first reported as type IV SCC mec (18) but was redefined as type VI SCC mec (4B) (19).

CLASSIFICATION OF SCC mec TYPES INTO SUBTYPES

Many different structures, including insertion sequences and transposons, have been identified among the major SCC mec types in regions other than the mec gene complex and ccr gene complex, i.e., in the J regions. Each SCC mec type has therefore been further classified into subtypes based on the polymorphisms or variations in J regions within the same ccr gene complex and mec gene complex combination. Table 2 lists the SCC mec types/subtypes reported from S. aureus with major differences in the J regions for which either the entire or partial nucleotide sequences have been reported.

To avoid potential misclassification of new SCC mec elements into types or subtypes, reporting novel SCC mec elements should be based on the entire nucleotide sequence of the element and not simply on PCR-based product sizes, which may be misleading.

Thus, novel SCC mec subtypes should be defined by the presence of specific DNA sequences located in J regions, including (i) characteristic genes, pseudogenes, or noncoding regions in J regions other than mobile genetic elements; and (ii) mobile genetic elements, e.g., insertion sequences, and plasmids or transposons, most of which encode antimicrobial resistance or other determinants.

To date, the following three methods have been used to describe subtypes of SCC mec elements: (i) expressing the J1 region differences as small letters, e.g., IVa, IVb, and IVc; (ii) expressing the differences due to the presence or absence of mobile genetic elements as capital letters, e.g., IA, IA, and IVA; and (iii) expressing the differences in each J1, J2, and J3 region in Arabic numbers, which are given in the order of discovery, e.g., II.1.1.1, II.1.1.2, and II.2.1.1.

Since the first and second nomenclature systems have been used as markers for particular epidemic clonal lineages, the designations have been retained as generic names.

However, to cope with the increasing diversity of J regions of SCC mec elements being reported, the number of alphabetic letters will be limited. Therefore, we are developing a computer system to identify or specify the differences in J regions based on a binary system (i.e., the presence or absence of specific DNA regions) that has been developed by Stephens et al. (21). The system, which will be provided on a dedicated website (http://www.SCCmec.org [under construction]), will be helpful in delineating the differences in J regions among elements more clearly and will identify the sets of genetic markers needed to differentiate the elements efficiently. Once the com-
FIG. 3. Phylogenetic relationships among ccrA genes, ccrB genes, and ccrC genes. The nucleotide sequences of 37 ccr genes (14 ccrA genes, 14 ccrB genes, and 9 ccrC genes) (see Table S1 in the supplemental material) were aligned by using the ClustalX program. In parentheses, names of species as well as strains are indicated in parentheses. A phylogenetic tree was generated using the neighbor-joining method by creating 2,000 bootstrap replicates. The tree was visualized with TreeView software, which was obtained from the TreeView website (http://taxonomy.zooology.gla.ac.uk/rod/treeview.html).

**COMPOSITES OF TWO OR MORE SCC ELEMENTS**

Recently, SCCmec elements carrying two ccr gene complexes have been identified (Fig. 1). For example, the SCCmec carried by *S. aureus* strain ZH47 is composed of an SCC with ccrC and an SCCmec with a class B2 mec gene complex (a subclass of class B mec gene complex into which a transposon Tn4001 was integrated), a type 2 ccr gene complex, and a J1 region with homology to type Ivc SCCmec (6). Although the two SCC elements are arranged in tandem, no characteristic DR sequence corresponding to ISS was identified at the junction regions, but two DR sequences were identified at the extremities of the composite element, suggesting that it is a single SCCmec containing two ccr gene complexes. Other examples include the SCCmec elements carried by Taiwanese *S. aureus* strains TSGH17 and PM1 (3, 22). These SCCmec elements are composed of an SCC with ccrC1 allele 2 and an SCCmec with a class C2 mec gene complex, a type 5 ccr gene complex carrying ccrC1 allele 2, and J1 regions specific to the SCCmec and demarcated by two DR sequences at both extremities. When a composite SCC element carrying two ccr genes is identified, the association of ccr genes, mec gene complexes, and J regions in the composite should be compared to those described previously in order to identify if it harbors any extant type of SCCmec. Following this, the association of the SCCmec element with the other ccr gene should be determined in order to establish whether the presence of the two ccr genes is a result of two separately integrated SCC elements and/or the composite was generated by the fusion of the two elements following deletion of the original junction region containing the DR in ISS.

Ultimately, the element in *S. aureus* ZH47 was classified as a type IV SCCmec element (2B&5), and the elements in *S. aureus* strains TSGH17 and PM1 were classified as type V SCCmec elements (5C2&5), although that of PM1 had been tentatively reported as type VII SCCmec (22). In line with the proposed criteria, the SCCmec carried by *S. aureus* strain HU25, which was reported as type IIIA SCCmec, should be regarded as a composite of two SCC elements, SCCChg and type III SCCmec, because the region carrying characteristic nucleotide sequences at the junction of the two elements was deleted and only two DR sequences, one downstream of orfX and the other at the right end of type III SCCmec, could be detected (18).

It is likely that many such composite elements will be discovered since these deletions of the original junction region seem to occur frequently. It is difficult to discriminate the presence of composite elements from the presence of structures carrying two elements in tandem using the current PCR strategies for SCCmec typing. Thus, novel elements carrying two ccr genes should not be given a roman numeral as a novel "type," but rather should be categorized as an SCCmec type variant based on the known type of SCCmec present in the composite element.

**CLASSIFICATION OF SCC ELEMENTS THAT DO NOT CARRY mecA**

Interestingly, SCC elements that do not carry mecA but contain other characteristic genes (e.g., capsule gene cluster, fusidic acid resistance, or the mercury resistance operon) have also been identified in staphylococci. These elements share the following characteristics with SCCmec: carriage of a ccr gene(s) (ccrAB and/or ccrC) in a ccr gene complex, integration at ISS in the staphylococcal chromosome, and the presence of flanking DR sequences containing the ISS.

We recommend that SCC elements be described by adding the suffix describing the genes’ names or their functions after SCC. For example, SCCcap1 carries the type 1 capsule gene cluster, SCCfur carries fusidic acid resistance, and SCCHg carries the mercury resistance operon. If no genes with inferable functions are found in the SCC, we recommend describing the SCC elements by adding the name of the strain, e.g., SCC_ZH47.

In addition, staphylococci can also harbor SCC-like regions similar to SCC that are integrated at ISS and bracketed by ISS but differ from SCC in that they do not harbor a ccr gene(s). They are diverse in size, from the shortest (0.1 kb) to the longest (34 kb), and have been described in different ways, e.g., SCC-like elements, an arginine catabolic mobile element, a...
**TABLE 2. SCCmec elements identified in MRSA**

<table>
<thead>
<tr>
<th>SCCmec type</th>
<th>Reported name</th>
<th>Major characteristics of J regions</th>
<th>Representative strain(s)</th>
<th>Reference(s) and/or accession no.</th>
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<td>I (1B)</td>
<td>I</td>
<td>J1, subtype 1-specific ORFs (pls); J3, dcs</td>
<td>NCTC10442, COL</td>
<td>9, 18</td>
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<td>L2</td>
<td>J1, subtype 2-specific ORFs; J3, dcs and pUB110</td>
<td>PL72</td>
<td>18</td>
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<td>II (2A)</td>
<td>II</td>
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<td>18</td>
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<td>JCSC3063</td>
<td>7</td>
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<tr>
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<td>IIb</td>
<td>J1, subtype 3-specific ORFs; J2, subtype 1-specific ORFs; J3, dcs and pUB110</td>
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<td>20</td>
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<td>IIE</td>
<td>J1, subtype 3-specific ORFs; J2, short J2 region the same as subtype 1 and Tn554; J3, dcs and pUB110</td>
<td>AR13.1/3330.2</td>
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<td>II.4.1.1</td>
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<td>III (3A)</td>
<td>III</td>
<td>J1, subtype 1-specific ORFs; J2, subtype 1-specific ORFs and Tn554; J3, subtype 1-specific ORFs and pT181</td>
<td>85/2082, ANS46</td>
<td>9, 18</td>
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<td></td>
<td>IIIA</td>
<td>J1, subtype 1-specific ORFs; J2, subtype 1-specific ORFs and Tn554; J3, subtype 1-specific ORFs, pT181, and SCCHg carrying ccrC</td>
<td>HCUS</td>
<td>18</td>
</tr>
<tr>
<td>IV (2B)</td>
<td>IVa</td>
<td>J1, subtype 1-specific ORFs; J3, dcs</td>
<td>CA05, MW2</td>
<td>15</td>
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<tr>
<td></td>
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<td>8/6-3P</td>
<td>15</td>
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<td>IVc</td>
<td>J1, subtype 3-specific ORFs; J3, dcs and Tn4001</td>
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<td>16</td>
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<td>M03-68</td>
<td>14</td>
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<tr>
<td></td>
<td>IVh</td>
<td>J1, subtype 6-specific ORFs; J3, dcs</td>
<td>EMRSA-15</td>
<td>17</td>
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<td>IVi</td>
<td>J1, subtype 7-specific ORFs; J3, dcs</td>
<td>JCSC6668</td>
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<td></td>
<td>IVj</td>
<td>J1, subtype 8-specific ORFs; J3, dcs</td>
<td>JCSC6670</td>
<td>2</td>
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<tr>
<td>IV (2B&amp;5)</td>
<td>IV variant</td>
<td>J1, subtype 3-specific ORFs; J3, SCC carrying ccrC</td>
<td>ZH47</td>
<td>6</td>
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<td>V (5C2)</td>
<td>V</td>
<td>J1, subtype 1-specific ORFs; J2, subtype 1-specific ORFs; J3, subtype 1-specific ORFs</td>
<td>WIS(WBG8318)</td>
<td>10</td>
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<tr>
<td>V (5C2&amp;5)</td>
<td>VT, VII</td>
<td>J1, subtype 2-specific ORFs; J2, subtype 2-specific ORFs; J3, SCC carrying ccrC</td>
<td>TSGH17, PM1</td>
<td>3, 22</td>
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<tr>
<td>VI (4B)</td>
<td>VI</td>
<td>J1, subtype 1-specific ORFs; J3, dcs</td>
<td>HDE288</td>
<td>19</td>
</tr>
<tr>
<td>VII (5C1)</td>
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<td>J1, subtype 1-specific ORFs; J2, subtype 1-specific ORFs; J3, subtype 1-specific ORFs</td>
<td>JCSC6082</td>
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<td>C10682, BK20781</td>
<td>23; FJ670542</td>
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</tbody>
</table>

* SCCmec elements that satisfy the following criteria are listed as follows: entire regions of SCCmec elements have been sequenced, or the essential parts of the mec gene complex and the ccr gene complex have been fully sequenced.

* The first categories for classifying SCCmec elements. The combinations of the ccr gene complex and the mec gene complex carried by the elements are indicated in parentheses.

* The names reported in original literature are listed. Assigned numbers or names that specify these elements would be given by IWG-SCC and would be seen at the website (http://www.SCCmec.org [under construction]).

* Key loci are indicated in parentheses. pls, plasmin-sensitive surface protein; dcs, downstream constant segment; kdp, potassium-dependent ATPase operon.

* GenBank/EMBL/DDBJ accession numbers are indicated in cases of direct submission.

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cassette chromosome, or an SCCmec insertion site genomic sequence. We suggest describing these elements as pseudo-SCC elements (ψSCC). We recommend that these ψSCC elements be designated by adding the suffix describing the genes’ names or their functions or by adding the name of the strain, similar to the case of SCC. In some cases, these ψSCCs are integrated in tandem with the SCCmec element as a region of a large cluster of foreign DNA in genome-sequenced staphylococcal strains.

**RECOMMENDATIONS AND CONCLUSIONS**

Researchers are encouraged to determine the entire nucleotide sequence of any putative novel SCCmec elements. It is also strongly recommended that all researchers about to submit a paper reporting a novel type of SCC element consult one of the members of the IWG-SCC in order to determine the most appropriate name for the new type. The working group will provide a Web page dedicated to the SCCmec element.
with updated information on SCCmec elements, a detailed classification system based on the differences in J regions, and currently available typing methods at http://www.staphylococcus.net and/or http://www.SCCmec.org/ (under construction). We hope that all researchers refer to this website to obtain relevant information on the epidemiological characterization of the SCCmec element.

Evaluation of the appropriate PCR-based strategies to be used in the epidemiological identification of SCCmec elements and the classification of SCC elements carried by staphylococcal strains other than those of S. aureus is under discussion and will be addressed in the future.

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