Two variants of staphylococcal cassette chromosome mec type IVA in community-associated meticillin-resistant Staphylococcus aureus strains in South Korea

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Meticillin-resistant Staphylococcus aureus (MRSA) strains harbouring staphylococcal cassette chromosome mec (SCCmec) type IVA are known to be more prevalent in South Korea than in other countries. Variations in the SCCmec IVA structure have been identified, including in sequence type (ST) 1 and ST72 strains. This study compared and investigated the genetic characteristics of two subtypes common in South Korea. Type IVA SCCmec of ST1 strains was characterized by type IV features with the linearized pUB110 at the junkyard (J) 3 region. However, that of ST72 strains carried a variant class B mec complex, ccrA2, with an identity of ~96% and the linearized pUB110 at the J3 region. In SCCmec of ST72 strains, the organization of the class B variant and the J3 region may be more similar to that of type IA than to other types, but the ccr type and other J regions seemed to be derived from type IV. These genetic characteristics showed that type IVA appears to result from the dynamic genetic exchange and recombination of SCC DNA.

INTRODUCTION

Staphylococcus aureus is a versatile opportunistic pathogen, with a variety of factors that are associated with virulence (Dinges et al., 2000; Lina et al., 1997; Novick, 2000), causing benign skin and soft tissue infections, as well as fatal invasive disease.

The key to the appearance of meticillin-resistant S. aureus (MRSA) is the acquisition of mecA encoding PBP2A, which has been shown to be part of a mobile element referred to as staphylococcal cassette chromosome mec (SCCmec) (Hartman & Tomasz, 1984; Ito et al., 1999, 2003). The main types of SCCmec are defined by the combination of the mec complex with the ccr locus (Ito et al., 2001, 2004; Okuma et al., 2002; Oliveira et al., 2006a; Takano et al., 2008). New types and subtypes have been reported, with diverse recombination of the mec complex, the ccr types and the junkyard (J) regions (Chongtrakool et al., 2006; Heusser et al., 2007; Hisata et al., 2005; Ito et al., 2003; Shore et al., 2005).

Community-associated (CA)-MRSA strains have emerged as a major concern with regard to MRSA infections (Etienne, 2005; Gillet et al., 2002; Ho et al., 2004; Tristan et al., 2007; Vandenesch et al., 2003). However, in South Korea, there is limited information on the emergence of CA-MRSA strains. A recently reported study on MRSA in South Korea during 2004–2005 showed that there were two prevalent CA-MRSA clones [clonal complex (CC) 1 and sequence type (ST) 72] that carried type IVA SCCmec (Park et al., 2007). The multiplex type IVA was first reported by Oliveira & de Lencastre (2002). In the multiplex pattern (downstream common sequence, pUB110 locus and mecA are amplified), type IVA appears to be related to IA, II and IV. Shore et al. (2005) reported multiplex type IVA carrying class A mec complex variants. However, type IVA found in South Korea was reported to harbour class B mec complex or variants (Park et al., 2007). Chongtrakool et al. (2006) proposed a new nomenclature for SCCmec type in...
which type IVA was described as 2B.N2; however, the genetic variations in the class B mec element, the ccr type and the J regions were not fully examined, and the authors stated that the data for the mec class and ccr type had been given tentatively.

Type IVA SCCmec has not been classified clearly into types and subtypes to date. Here, we have described the genetic characteristics of type IVA found in South Korea and investigated its genetic relationship with other types. We have also reported two variants of type IVA.

METHODS

Bacterial strains. For analysis of the two subtypes of SCCmec IVA, we used strains cm11 ST72 and cm14 (ST1); these STs have frequently been detected in CA-MRSA strains in South Korea (Park et al., 2007). For the comparative study of the distribution of aminoglycoside-resistance genes and virulence-associated genes, we used 23 isolates of S. aureus including virulence and antibiotic-resistance patterns are summarized in Table 1. Some of the SCCmec PCR typing and antibiotic susceptibility results were included in the findings of a previous study (Park et al., 2007). The profile of the enterotoxins showed that SCCmec isolates carried seh (>95 %) and ST72 carried an efg variant harbouring seu2. There were different antibiotics-susceptibility patterns in ST72 and CC1 strains, especially for gentamicin (Table 1); this was probably due to the presence of SCCmec identified in ST72 and CC1 strains (Table 1).

Profiling of virulence-associated genes, aminoglycoside resistance-associated genes and antimicrobial susceptibilities. Enterotoxin and surface protein encoding genes, and aminoglycoside-resistance-associated genes from 55 isolates of CC1 and ST72 were evaluated as described by Choi et al. (2003), Jarraud et al. (2002) and Vancraeynest et al. (2004). Variation in the enterotoxin gene island (egc) was determined as described by Thomas et al. (2006), Antimicrobial susceptibilities were determined using the disc diffusion method, as recommended by the Clinical and Laboratory Standards Institute (CLSI, 2006).

Nucleotide sequence accession numbers. Nucleotide sequences determined in the present study were deposited in GenBank (http://www.ncbi.nlm.nih.gov/Genbank/) under accession numbers EU437549 (the complete SCCmec sequence of the ST72 clone) and EU437550 (the mec element sequence of the ST1 clone).

RESULTS AND DISCUSSION

Molecular characteristics and antibiotic susceptibility of ST72 and CC1 isolates

Recently, Kim et al. (2007) and Park et al. (2007) reported that multiplex type IVA was found in ~43 and ~53 % of CA-MRSA isolates in South Korea, respectively. In their studies, type IVA was the most prevalent type in CA-MRSA and was found mainly in clinical isolates belonging to ST72 and CC1 (Kim et al., 2007; Park et al., 2007). Their characteristics including virulence and antibiotic-resistance patterns are summarized in Table 1. Some of the SCCmec PCR typing and antibiotic susceptibility results were included in the findings of a previous study (Park et al., 2007). The profile of the enterotoxins showed that SCCmec isolates carried seh (>95 %) and ST72 carried an efg variant harbouring seu2. There were different antibiotics-susceptibility patterns in ST72 and CC1 strains, especially for gentamicin (Table 1); this was probably due to the presence of aac(6')-aph(2') in CC1 isolates (Table 1). Although both clones (ST72 and CC1) carried ant(4') at the pUB110 locus of SCCmec IVA (Table 1), ANT(4')-Ie encoded by ant(4') is not related to gentamicin resistance but is associated with resistance to amikacin, tobramycin, dibekacin, isepamicin and kanamycin (Vakulenko & Mobashery, 2003). However, AAC(6')-Ie--APH(2')-Ie encoded by aac(6')-aph(2') confers resistance to virtually all aminoglycosides including gentamicin, but not for streptomycin (Vakulenko & Mobashery, 2003).

Genetic variation of SCCmec type IVA

There were some variations in the PCR results for class B mec and ccrAB identified in ST72 and CC1 strains (Table 1). We compared the class B mec complex and ccrAB...
Table 1. Molecular characteristics and antibiotic-resistance patterns of major SCCmec IVA clones

| MLST | PFGE pattern | SCCmec multiplex type* | mec class† | ccr type‡ | J region§ | agr type§ | luk-PV|| | hlg∥ | Toxin|| | MSCRAMMs¶ | AME# | Antibiotic resistance (%)** |
|------|--------------|------------------------|------------|-----------|-----------|-----------|-------------|-----|-------|-------|-----------------|------|-------------------|
|      |              |                        |            |           | IVc, pUB110 | I        | hlg2        | egc | icaA-icaD-ephS-frnA-eno-clfA-clfB-fib | ant(4’) | 3.1 3.1     | 50.0 | 0 | 100                |
| ST72 | B            | IVA                    | (B)††      | (2)‡‡     | IVc, pUB110 | III      | hlg2        | sch | icaA-icaD-ephS-frnA-cna-eno-clfA-clfB-fib | ant(4’), aac(6’)/aph(2’)| 100 0 | 94.7 | 57.9 | 100               |
| CCI§§| D            | IVA                    | B          | 2         |           |           |             |     |                                  |                  |       |      |      |               |

CLI, clindamycin; ERY, erythromycin; GEN, gentamicin; TET, tetracycline; TOB, tobramycin.

*Multiplex PCR results described by Oliveira & de Lencastre (2002).
†Single PCR results described by Okuma et al. (2002).
‡Subtyping of the J region was performed as described by Hisata et al. (2005); other characteristics were determined from multiplex PCR results.
§Accessory gene regulator locus (agr) group was determined by restriction enzyme DraI RFLP analysis, as described by Papakyriacou et al. (2000).||PVL, hlg, hlg-2 and other toxin-related genes were determined as described by Jarraud et al. (2002).
¶Genetic profile of microbial surface components recognizing adhesive matrix molecules (MSCRAMMs). The bold text indicates the difference between the profiles for ST72 and ST1.
#AME, aminoglycoside-modifying-enzyme-associated gene.
**All isolates showed resistance to oxacillin and penicillin, but no isolate showed resistance to arbekacin, ciprofloxacin, rifampicin, penicillin or vancomycin.
††Increased size of class B mec.
‡‡The ccrA2 gene shared 96% identity with the other ccrA2 gene.
§§CCI: 21/23 ST1, 1/23 ST493 and 1/23 ST573.
variations in cm11 (ST72) and cm14 (ST1) strains. The overlapping single SCC mec PCR products were sequenced to compare the differences in more detail (Fig. 1). Variations were found in the class B mec complex of strain cm11: a transposase 20 family gene (tnp20, OR F C 0 1 7 ; Table 2) was inserted, and the hypervariable region (HVR) and ugpQ were deleted, when compared with other class B mec elements (Fig. 1). tnp20 was almost identical (~99.6 %) to SCC pbp4, commonly reported in Staphylococcus epidermidis strains; it has also recently been reported in a Swedish MRSA strain (JCSC6668; Berglund et al., 2009). The class B.3 mec, of strain JCSC6668, was similar to that of strain cm11, except for deletion of the HVR and ugpQ (Fig. 1). The deletion of the HVR was similar to the class B mec element of type IA (Fig. 1).

In contrast, strain cm14 (ST1) carried a typical class B mec (IS1272–D mecR1–mecA–HVR–IS431), when compared with other SCC mec IV types (Fig. 1). It had the mecA upstream region, similar to SCC mec type IVc, but had linearized plasmid pUB110 in the downstream region, which was different from that of IVc (Table 1).

ccr allotyping has been useful for examination of the phylogenetic relationships among SCC mec types (Oliveira et al., 2006b, 2008). Therefore, we investigated the genetic

Table 2. List of ORFs of SCC mec type IVA from the ST72 clone

<table>
<thead>
<tr>
<th>ORF</th>
<th>Position (nt)</th>
<th>Identity (%)</th>
<th>Homologues and other characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>IR-L</td>
<td>3238–3263</td>
<td>–</td>
<td>Inverted complementary repeat (IR-L) of SCC mec</td>
</tr>
<tr>
<td>C007</td>
<td>3303–4103</td>
<td>99</td>
<td>Hypothetical protein (S. aureus MR108, SCC mec IVc), abortive phage resistance protein (S. epidermidis ATCC 12228)</td>
</tr>
<tr>
<td>C008</td>
<td>4133–5287</td>
<td>100</td>
<td>Hypothetical protein (S. aureus MR108), abi-alpha protein (S. epidermidis ATCC 12228), COG2865</td>
</tr>
<tr>
<td>C009</td>
<td>5742–6788</td>
<td>100</td>
<td>Hypothetical protein (S. aureus MR108), hypothetical protein (S. epidermidis ATCC 12228)</td>
</tr>
<tr>
<td>C010</td>
<td>7277–9070</td>
<td>99</td>
<td>Hypothetical protein (S. aureus MR108), pfam06048 (DUF927)</td>
</tr>
<tr>
<td>ccrA2</td>
<td>9304–10653</td>
<td>96</td>
<td>Cassette chromosome recombinase A type 2 (S. aureus MR108)</td>
</tr>
<tr>
<td>ccrB2</td>
<td>10675–12303</td>
<td>100</td>
<td>Cassette chromosome recombinase B type 2 (S. aureus MR108)</td>
</tr>
<tr>
<td>C013</td>
<td>12825–13175</td>
<td>100</td>
<td>Hypothetical protein (S. aureus MR108, SCC mec IVc)</td>
</tr>
<tr>
<td>C014</td>
<td>13262–13573</td>
<td>100</td>
<td>Hypothetical protein (S. aureus MR108, SCC mec IVc)</td>
</tr>
<tr>
<td>C015</td>
<td>13585–14094</td>
<td>100</td>
<td>Hypothetical protein (S. aureus MR108, SCC mec IVc), COG4333</td>
</tr>
<tr>
<td>IS1272</td>
<td>14230–15741</td>
<td>98</td>
<td>Truncated transposase of IS1272, COG3666 (transposase 11)</td>
</tr>
<tr>
<td>C017</td>
<td>15838–16851</td>
<td>99</td>
<td>Transposase (S. epidermidis ATCC 12228), pfam02371 (transposase 20)</td>
</tr>
<tr>
<td>ΔmecR1</td>
<td>17536–18504</td>
<td>100</td>
<td>Truncated signal transducer protein MecR1</td>
</tr>
<tr>
<td>mecA</td>
<td>18622–20628</td>
<td>100</td>
<td>Penicillin-binding protein 2</td>
</tr>
<tr>
<td>IS431</td>
<td>21035–21709</td>
<td>100</td>
<td>Transposase for IS-like element (IS431 mec), COG3316</td>
</tr>
<tr>
<td>C021</td>
<td>21960–22673</td>
<td>99*</td>
<td>Kanamycin nucleotidyl transferase (pUB110)</td>
</tr>
<tr>
<td>C022</td>
<td>22896–23294</td>
<td>100</td>
<td>Bleomycin resistance protein (BRP), pfam09093 (gloxylalase)</td>
</tr>
<tr>
<td>C023</td>
<td>23801–25063</td>
<td>99</td>
<td>Plasmid recombination enzyme (plasmid pUB110), pfam01076</td>
</tr>
<tr>
<td>C024</td>
<td>25287–26081</td>
<td>100</td>
<td>Replication protein (plasmid pUB110), COG5655</td>
</tr>
<tr>
<td>IS431</td>
<td>26382–27056</td>
<td>100</td>
<td>Transposase for IS-like element (IS431 mec), COG3316</td>
</tr>
<tr>
<td>C026</td>
<td>27088–27327</td>
<td>100</td>
<td>Hypothetical protein SAV0026 (S. aureus Mu50)</td>
</tr>
<tr>
<td>C027</td>
<td>27742–29037</td>
<td>100</td>
<td>Hypothetical protein SAV0025 (S. aureus Mu50)</td>
</tr>
<tr>
<td>IR-R</td>
<td>29310–29335</td>
<td>–</td>
<td>Inverted complementary repeat (IR-R) of SCC mec</td>
</tr>
<tr>
<td>orfX</td>
<td>29320–29806</td>
<td>–</td>
<td>ORF X partial</td>
</tr>
</tbody>
</table>

*Result from BLASTN.
variation and relationship of ccrAB of type IVA. The nucleotide sequence of the ccrAB locus in strain cm14 was almost identical (99%) to that of the type IVc variant. The ccrB2 sequence from strain cm11 was identical (100%) to that of IVc (MR108); however, ccrA2 was slightly different, with a 96% identity determined by BLASTP. Comparison of type IVA and other ccrA alleles showed that most of the type IV subtypes clustered in one closely related group distinct from those of type II, with the exception of type IVb (Fig. 2). The ccrA2 of type IVA (cm11) clustered with type IV subtypes and was much closer to that of SCCmec IVg (Fig. 2). In the nucleotide sequence analysis of ccrA2, type IVA should be phylogenetically closer to type IV than to type II.

**SCCmec type IVA of strain cm11 (ST72) could provide evidence of exchange and recombination of SCC DNA in staphylococci inter- and intra-species**

We examined the whole SCCmec structure of strain cm11 (ST72), which appeared to be similar to SCCmec type 2B,N,2 (Fig. 3a). Twenty-one ORFs in SCCmec were identified by ORF Finder and BLASTP; most had >98% identity with previously identified staphylococcal genes, except for ccrA2 (Table 2). The dispersed ORFs of the left extremity region were almost identical to those of type IVc (MR108) and SCCpbb4 (S. epidermidis ATCC 12228) (Fig. 3b, Table 2). The J3 region with linearized pUB110 was almost identical to that of IVc (MR108); however, ccrA2 and the J3 region in this structure may be more similar to that of type IA and II (Table 2). The dispersed ORFs of the left extremity region of SCCmec were almost identical to those of type IVc (MR108) and SCCpbb4 (S. epidermidis ATCC 12228) (Fig. 3b, Table 2). The J3 region with differentiated pUB110 was almost identical to that of type IA and II (Table 2). The organization of the class B variant and the J3 region in this structure may be more similar to that of type IA and other types, but the ccrA2 and other J regions seemed to be derived from type IV (Fig. 3a). Analysis of type IVA showed a unique structure composed of the recombination of a variant class B mec element and the type 2 ccrAB with the J regions derived from type IA or II (J3) and IVc or SCCpbb4 (J1 and J2) (Fig. 3a). It has been suggested that exchange and recombination of SCC DNA probably occurs in staphylococci inter- and intra-species (Hanssen & Sollid, 2007; Hanssen et al., 2004). The genetic structure of SCCmec type IVA, from strain cm11, could provide evidence supporting both inter- and intra-species recombination.

**Analysis of the left boundary of SCCmec IVA indicates SCCmec acquisition by non-CcrAB-mediated insertion**

The integration sequence and the inverted repeats at the right and left boundaries of SCCmec were identical to SCCmec type IVc (S. aureus MR108). However, the outermost sequence of the SCCmec attachment site (SCCmec attB) of the left boundary was very different from that of IVc (MR108) (Fig. 3c). The results of primer-walking studies showed that the 15 bp core sequence (SCCmec attB) and the SCCmec acquisition site of strain 15666 (MSSA ST72) were conserved surrounding the sequences at the left-end extremity of SCCmec IVA (cm11) (Fig. 3a, c). Noto et al. (2008) suggested that there could be two mechanisms for the acquisition of SCCmec; CcrAB-mediated and non-CrAB-mediated insertion mechanisms (other integrase mechanisms or homologous recombination). For CcrAB-mediated acquisition, a specific sequence should be necessary at the surrounding boundary of the left-end extremity. However, at the left-end extremity of type IVA, there was no CcrAB-mediated specific sequence (Fig. 3c). In addition, there were no lost or gained sequences at the SCCmec acquisition site, compared with strain 15666 (Fig. 3c). These findings suggest that MSSA ST72 strains could acquire SCCmec type IVA by non-CrAB-mediated insertion.

**Epidemiological features of SCCmec type IVA strains prevalent in South Korea**

The genetic diversity of CA-MRSA strains is probably due to the successful conversion of diverse MSSA lineages to MRSA by the transfer of SCCmec in the community. In particular, SCCmec type IV, the smallest structural and most diverse type, predominates among the diverse CA-MRSA strains worldwide (Robinson & Enright, 2003; Vandenesch et al., 2003). However, instead of type IV, SCCmec IVA types are the most prevalent CA-MRSA strains found in South Korea, compared with other countries (Cha et al., 2005; Kim et al., 2007; Park et al., 2007, 2008). The emergence of SCCmec type IVA strains is a major concern in the public health setting and for infection control in hospitals, and these strains have recently been shown to be spreading (Kim et al., 2007; Park et al., 2007, 2009).

Epidemiological studies of S. aureus and antibiotic use in South Korea have reported that ST72 (major SCCmec IVA
Fig. 3. Schematic analysis of ST72 SCCmec type IVA. (a) The genetic structure of IVA is based on the nucleotide sequences deposited in GenBank under accession numbers EU437549 (SCCmec of ST72 clone) and EU272085 (MSSA ST72 15666 clone). ORFs are indicated by arrows. The bars indicate homology to previously described SCCmec elements. (b) The homologous left-extremity ccr (L-C) region of SCC elements. ORFs of SCCmec IVA are indicated by boxes. Each homologous ORF of IVc and SCCpbp4 is described below the boxes. The nucleotide sequences with GenBank accession numbers EU437549 (cm11 SCCmec IVA), AE016744 (S. epidermidis ATCC 12228 SCCpbp4) and AB096217 (strain MR108 SCCmec type IVc) were used. (c) Comparison of the chromosome–SCCmec junction sequences of S. aureus strain cm11. The nucleotide sequences around the left (i) and right (ii) boundaries were aligned with those of strains MR108 (type IVc, AB096217), M03-68 (type IVg, DQ106887), N315 (type II, D86934) and the SCCmec insertion site of MSSA strain 15666 (EU272085). The dotted arrows indicate inverted repeats of IR-L and IR-R at both extremities of the SCCmec elements. The box indicates the SCCmec attB site.
clonal lineage) MSSA and MRSA strains were not frequently isolated until 2005 in South Korea (Kim et al., 2008). This suggests that IVA strains have emerged recently in South Korea. In addition, it has been reported that the antimicrobial use density of aminoglycosides was one of the highest in a survey of antimicrobial use during 2004–2007, which corresponded with the results of the Health Insurance Review and Assessment Service (http://www.hira.or.kr) in South Korea (Yoon et al., 2008). The finding that ant(4′) was carried on the genetic island of type IV IVA by both SCCmec IVA strains (ST72 and CC1) and that aac(6′)/aph(2′) was carried by CC1 strains (Table 1) suggests that exposure to aminoglycosides might provide a favourable environment for the spread of SCCmec IVA strains rather than IV strains.

In conclusion, we have described two variants of SCCmec IVA, each of which showed genetic differences in the class B mec complex. In particular, the genetic organization of type IV IVA in ST72 strains appeared to be more related structurally to types IA (class B mec complex variation and J2 region) and IV (ccr type, J1 and J2 region) than the other types. The genetic relationship of ORFs on SCCmec type IVA suggests that the development of type IVA appears to result from the dynamic genetic exchange and recombination of SCC DNA inter- and intra-species. In the future, it will be necessary to investigate the genetic evolutionary relationships of other SCC DNAs, as well as their epidemiology, to determine how these types disseminate in community and hospital settings.

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