Characterization of SCCmec type IV methicillin-resistant *Staphylococcus aureus* clones increased in Japanese hospitals

Hidemasa Nakaminami,1 Shunsuke Takadama,1,2 Ayumu Ito,1 Mariko Hasegawa,1 Chika Jono,1 Miyuki Noguchi,1 Manami Shoshi,1 Takeaki Wajima,1 Takeshi Fujii,2 Hiroshi Maruyama,3 Haruo Sakamoto,4 Yutaka Ito,5 Satsuki Okamoto,6 Yukiyoshi Masaki,7 Kayoko Tsuchiya,8 Susumu Nishinari9 and Norihisa Noguchi1,*

**Abstract**

Recently, the prevalence of staphylococcal cassette chromosome mec (SCCmec) type IV MRSA, which are the major community-acquired methicillin-resistant *Staphylococcus aureus* (MRSA), have increased in Japanese hospitals. The aim of this study was to elucidate the detailed molecular epidemiological features of the SCCmec type IV clones in Japanese hospitals. When 2589 MRSA isolated from four hospitals in Tokyo, Japan between 2010 and 2014 were analysed, the proportion of SCCmec type IV overtook that of type II, which was the major type of hospital-acquired MRSA in 2014. Multilocus sequence typing showed that CC1 was the most predominant clone in the SCCmec type IV isolates. The clinical departments that the patients belonged to, pulsed-field gel electrophoresis analysis and antimicrobial susceptibility profiles suggested that the origin of the CC1-SCCmec type IV (CC1-IV) clone was a community setting. Our data show that the CC1-IV clone is becoming a predominant MRSA clone in Japanese hospitals.

Since methicillin-resistant *Staphylococcus aureus* (MRSA) was first identified in the United Kingdom in 1961, it has spread worldwide [1]. Sequence type 5 staphylococcal cassette chromosome mec (SCCmec) type II (ST5-II) New York/Japan clones are found mainly in hospital-acquired MRSA (HA-MRSA) isolated in the USA, Korea and Japan [2, 3]. HA-MRSA frequently carries SCCmec type I, II, or III, which provide high-level resistance to multiple antimicrobial agents, including *β*-lactams, and often cause infectious diseases in immunocompromised hosts, especially hospitalized patients.

The prevalence of community-acquired MRSA (CA-MRSA) in healthy individuals with no risk for HA-MRSA infection is a major public health concern [4]. CA-MRSA frequently carries SCCmec type IV or V, is resistant to *β*-lactam antimicrobial agents only or to limited agents of other classes, and often produces Panton–Valentine leukocidin (PVL), which is associated with apoptosis and necrosis in human polymorphonuclear cells or monocytes [4]. CA-MRSA is mainly associated with skin and soft tissue infections, but also occasionally with invasive infections (such as bacteraemia and necrotizing pneumonia) in healthy individuals, including children and adolescents [5].

The predominant CA-MRSA isolated in the USA is the USA300 clone with the ST8-IV lineage [4]. The USA300 clone is PVL gene (*lukS/F-PV*)-positive and possesses the arginine catabolic mobile element (ACME), which enhances colonization and adhesion to the host [6]. In contrast, the ST59-IV, ST59-V, ST30-IV and ST30-V clones are the predominant CA-MRSA strains in Asia [3]. In Japan, in...
particular, the prevalence of lukS/F-PV-positive CA-MRSA, such as the USA300 clone, is very limited [7]. However, ST8-IV clones, including USA300, have been recently isolated in both Japan and Korea [8, 9].

Dissemination of HA-MRSA (ST5-II and ST239-III) in the community and CA-MRSA (ST8-IV and ST59-IV) in hospitals has been reported frequently in recent years [10, 11]. Similar data were reported in Japan [12, 13], although the MRSA isolates have not been monitored for a long period. Previously, we investigated the annual transition of SCCmec types in MRSA isolated in a single hospital located in Tokyo, Japan, for 8 years beginning in 2005 [14]. The proportion of SCCmec type II decreased significantly (from 90.0 to 74.3 %), while that of SCCmec type IV increased significantly (from 5.8 to 16.3 %). Additional reports also indicated that the proportion of SCCmec type IV isolates in hospitals was elevated in other areas of Japan [12, 13]. However, the characteristics of the SCCmec type IV isolates, which have increased in Japan, are still unknown. Here, we investigated the detailed molecular epidemiological features of these isolates. Our data suggested that the clonal change was caused by the emergence of a specific clonal complex (CC) 1-IV clone.

A total of 2589 MRSA isolates were collected from four hospitals located in Tokyo, Japan (Table S1, available in the online version of this article). All strains were isolated from different patients. S. aureus ATCC29213 was used as the quality control strain for antimicrobial susceptibility testing. The N315 (New York/Japan clone) was used as the reference DNA standard for pulsed-field gel electrophoresis (PFGE) analyses. JCSC6774 (USA300 clone) was used to compare the PFGE patterns [15]. All strains were cultured on tryptone soy agar (Oxoid, Hampshire, UK) under aerobic conditions at 35 °C for 24 h. S. aureus was identified as described previously [16]. Strains that could not be identified by PCR were determined by sequencing the 16S rRNA gene [17]. MRSA strains were identified by PCR-based detection of the meca gene [18]. Samples were prepared from a single MRSA colony suspended in 100 µl sterile water [19]. PCR assays for detecting the meca, tst (encoding toxic shock syndrome toxin-1), lukS/F-PV, ACME arcA and opp-3C genes, and SCCmec typing were performed as described previously [19–23]. Isolates not identified as SCCmec types I to V were classified as nontypeable (NT). Minimum inhibitory concentrations (MICs) were determined by an agar microdilution method, according to the Clinical and Laboratory Standards Institute guidelines [24]. The breakpoints of the antimicrobial agents were defined using the Clinical and Laboratory Standards Institute interpretation criteria [25]. The breakpoint of spiramycin was defined as described in a previous study [14]. PFGE was performed for Smal-digested chromosomal DNA as described previously [18, 23, 26]. The DNA patterns obtained by PFGE were analysed with BioNumerics software version 7.1 (Applied Maths, Saint-Martens-Latem, Belgium) using the Dice coefficient (optimization, 1.0 % band tolerance, 1.0 %). Major pulsotypes (A to H) were defined by more than four isolates exhibiting >75% similarity. Multilocus sequence typing (MLST) was performed as described previously [18, 27]. Differences in the rates of SCCmec types and antimicrobial resistance rates were tested by the χ² test or Fisher’s exact test (n<10).

The annual transitions of SCCmec types for 2589 MRSA isolates are shown in Fig. 1. The SCCmec types I, II, III, IV, V and NT were detected in 1.9 % (49 isolates), 61.8 % (1599 isolates), 0.2 % (6 isolates), 25.2 (653 isolates), 1.7 % (45 isolates) and 9.2% (237 isolates) of the total isolates, respectively. The proportion of SCCmec type II was 78.8 % (431 isolates) in 2010 and decreased significantly after 2011 (P<0.01), reaching 41.2 % (168 isolates) in 2014. In contrast, the proportion of SCCmec type IV increased significantly from 12.4 % (68 isolates) to 45.3 % (185 isolates) over this period. These phenomena were observed in all tested hospitals. In particular, a remarkable increased prevalence of SCCmec type IV isolates was observed in 2014 in all tested hospitals.

To elucidate the molecular epidemiological features of the SCCmec type IV isolates in 2014, we randomly selected 19.7 % (38/193 strains), 33.3 % (16/92 strains), 34.1 % (14/85 strains) and 61.9 % (13/38 strains) of the strains from hospitals A, B, C and D, respectively, and performed MLST analysis (Table 1). Three major clones (CC1, CC8 and CC81) were found. Notably, the CC1-IV clone was the most predominant clone in hospitals A, B and D, and was found in all tested hospitals. The detection rates for the CC8-IV and CC81-IV clones were above 10 %, but both clones were detected in only part of the tested hospitals. The clinical departments of patients associated with the three major clones are listed in Table S2. The patients from the Critical Care Center were the most associated with the CC1-IV and CC8-IV clones, while the CC8-IV clone was also associated with the Dermatology department.

To understand the detailed genetic features of the SCCmec type IV isolates, we performed PFGE analysis and detection of lukS/F-PV, tst and ACME (Fig. S1). In this experiment, 5 out of 81 strains could not be analysed because of their poor lysis. Sixty-eight (89.5 %) of them were divided into three major pulsotypes (A to C). Pulsotype A consisted of the CC8-IV clone, included the reference strain of USA300 and associated with lukS/F-PV and ACME type I. This indicated that the four strains of pulsotype A were USA300 clones. However, the other CC8-IV clone (pulsotype B) was positive for tst but not lukS/F-PV. The CC1-IV clone formed the most predominant pulsotype and did not carry the virulence genes tested in this study. Notably, the CC81-IV clones belonged to the same pulsotype as the CC1-IV clones, but they were divided into different clades. The specific CC1-IV clone belonging to pulsotype C was found in all tested hospitals, indicating that it was widely disseminated in hospitals in Tokyo, Japan.
The antimicrobial susceptibilities of the 3 major clones of SCCmec type IV (50 CC1-IV, 15 CC8-IV and 9 CC81-IV) isolates (Table 1) were compared (Table 2). The antimicrobial susceptibilities of the CC1-IV and CC8-IV clones were high and similar to those of typical CA-MRSA strains [23]. However, the resistance rates of the CC1-IV clone to levofloxacin and clarithromycin were higher than those of the CC8-IV clone. Remarkably, the CC81-IV clone exhibited high resistance rates and MIC$_{50}$/MIC$_{90}$ values (≥256 µg ml$^{-1}$) to multiple antimicrobial agents, like a typical HA-MRSA CC5-II New York/Japan clone [14].

The finding that the predominant MRSA clone in Japanese hospitals changed from SCCmec type II to IV isolates by 2014 indicates that the occurrence of type IV has increased, considering data from our previous study covering the period from 2005 to 2012 [14]. Other findings demonstrate that the prevalence of SCCmec type IV clones has increased in different hospitals located in northern and southern Japan [12, 13]. Therefore, the prevalence of SCCmec type IV clones in hospitals is increasing throughout Japan.

The CC1 clone was the most predominant clone of SCCmec type IV isolates in 2014. The CC1 consisted of ST1, ST2180, ST2725 and ST2764 strains. The ST1 strain is well known as the CA-MRSA lineage of the USA400 clone [28]. However, the CC1-IV clone has rarely been found in Japanese CA-MRSA [29, 30]. To the best of our knowledge, this is the first report showing that the CC1-IV clone occurred in...
Japanese hospitals. The CC1-IV clone was frequently found in patients of the Critical Care Center. This department is frequently associated with CA-MRSA [31]. Furthermore, the CC1-IV clone exhibited high antimicrobial susceptibility, like a typical CA-MRSA. These data strongly suggest that the origin of the CC1-IV clone might be a community setting. A typical USA400 clone is positive for lukS/PV, although all of the CC1-IV clones found in this study were lukS/PV-negative. This suggests that the CC1-IV clone found in this study is not a USA400 clone. Recently, CC1 MRSA clones have been found in livestock in other countries [32, 33]. Dissemination of the CC1 clones in hospitals has also been reported in Europe [34]. The precise origin of the CC1-IV clone found in this study is still unclear. However, increased prevalence of the CC1-IV clone was also found in another Japanese hospital in 2016 (Nakaminami et al., unpublished data), indicating that the CC1-IV clone that was widely disseminated in hospitals is becoming a predominant MRSA clone in Japanese hospitals.

The CC8-IV clone mostly consisted of ST8 strains. One of the CC8 clones (pulsotype B) frequently carried the toxic shock syndrome toxin gene, tst. This type of MRSA strain has already been reported as the ST8 CA-MRSA/J clone in Japan and is known as a causative agent of invasive and severe infections [35]. The CC8-IV clone was associated with patients in the Critical Care Center and the Dermatology department. Additionally, the CC8-IV clone exhibited high antimicrobial susceptibility, as did the CC1-IV clone. Furthermore, the CC8-IV clone (pulsotype A) belonged to the same pulsotype of the USA300 clone, which is a typical CA-MRSA clone. The data strongly suggest that the origin of the CC8-IV clone was a community setting. We previously reported that diverse CC8-IV clones (not only the

Table 1. Clonal complexes of SCCmec type IV clones isolated in 2014

<table>
<thead>
<tr>
<th>Clonal complex</th>
<th>Sequence type (n)</th>
<th>No. (%) of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Hospital A (n=38)</td>
</tr>
<tr>
<td>1</td>
<td>1 (24), 2180 (2), 2725 (3), 2764 (20), SLV (1)</td>
<td>24 (63.2)</td>
</tr>
<tr>
<td>5</td>
<td>5 (2), 764 (1)</td>
<td>1 (2.6)</td>
</tr>
<tr>
<td>8</td>
<td>8 (12), 770 (2), SLV (1)</td>
<td>10 (26.3)</td>
</tr>
<tr>
<td>30</td>
<td>30 (2), 3607 (1)</td>
<td>2 (5.3)</td>
</tr>
<tr>
<td>81</td>
<td>8325 (9)</td>
<td>0</td>
</tr>
<tr>
<td>Other</td>
<td>834 (1)</td>
<td>1 (2.6)</td>
</tr>
</tbody>
</table>

SLV, single-locus variant with no sequence match.

Table 2. Comparison of the antimicrobial susceptibilities of the three major SCCmec type IV clones identified in this study

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>CC1 (n=50)</th>
<th>CC8 (n=15)</th>
<th>CC81 (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC&lt;sub&gt;90&lt;/sub&gt; / MIC&lt;sub&gt;99&lt;/sub&gt;</td>
<td>R (%)</td>
<td>MIC&lt;sub&gt;90&lt;/sub&gt; / MIC&lt;sub&gt;99&lt;/sub&gt;</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>16/32</td>
<td>100</td>
<td>16/32</td>
</tr>
<tr>
<td>Oxacillin</td>
<td>64/64</td>
<td>100</td>
<td>32/64</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>32/64</td>
<td>100</td>
<td>32/64</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>64/64</td>
<td>100</td>
<td>0.13/32</td>
</tr>
<tr>
<td>Clarithromycin</td>
<td>≥256/6256</td>
<td>94.0</td>
<td>0.13/256</td>
</tr>
<tr>
<td>Spiramycin</td>
<td>8/8</td>
<td>4.0</td>
<td>≥256</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>0.25/0.25</td>
<td>0</td>
<td>32/64</td>
</tr>
<tr>
<td>Minocycline</td>
<td>≤0.06/0.13</td>
<td>2.0</td>
<td>≤0.06/16</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>1/1</td>
<td>0</td>
<td>1/1</td>
</tr>
<tr>
<td>Linezolid</td>
<td>1/1</td>
<td>0</td>
<td>1/1</td>
</tr>
</tbody>
</table>

*P<0.05 versus CC1 and CC8, as determined by Fisher's exact test.
**P<0.05 versus CC8, as determined by Fisher's exact test.
***P<0.05 versus CC1, as determined by Fisher's exact test.
USA300 clone) are increasing in prevalence in the Japanese community [9], indicating that increase of the CC8-IV clone in the community has contributed to dissemination of the CC8-IV clone in hospitals.

The CC81-IV clone accounted for 11% of isolates in 2014. The CC81 consisted of the ST3235 strain. Notably, the antimicrobial resistance level of the CC81-IV clone was apparently higher than that of the other SCCmec type IV clones. Additionally, the antimicrobial-susceptibility pattern of the CC81-IV clone was identical to the CC5-II clone as reported previously [18]. The information regarding the CC81-IV clone is very limited, but there is a report indicating that it caused food poisoning outbreaks in Japan [36]. In this study, we identified a high-level multiple antimicrobial-resistant CC81-IV clone for the first time in Japanese hospitals. We should monitor this clone because it showed equivalent resistance to the New York/Japan clone despite being a SCCmec type IV clone.

Several limitations of this study should be acknowledged. Firstly, because the four hospitals in this study are located in a limited region of Japan, our data do not reflect the conditions in all regions of Japan. Secondly, we were not able to evaluate exact epidemiological data because some information concerning patients and their backgrounds was lacking. We could not obtain information regarding the admission dates and the dates of specimen collection. Therefore, the exact derivation of the SCCmec type IV isolates could not be determined. Thirdly, the SCCmec type IV strains that were examined by MLST and PFGE analyses did not cover all 2014 isolates. Therefore, the accurate proportions of CC1-IV, CC8-IV and CC81-IV clones could not be determined. However, we predict that the CC1-IV clone is becoming a predominant HA-MRSA in Japan, because we have observed similar phenomena in other hospitals (data not shown).

In conclusion, our study revealed that SCCmec type IV clones, particular the CC1-IV clone in particular, have spread in hospitals in Japan. The CC1-IV clone has the potential to take the place of traditional SCCmec type II clones in a hospital setting. Such a multidrug-resistant SCCmec type IV clone will cause more complicated diseases and become a serious threat for immunocompromised patients in a healthcare setting. Therefore, more strict precautions against contact infection are necessary to prevent dissemination of the SCCmec type IV clones.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

Ethical statement
The study protocol was approved by Tokyo University of Pharmacy and Life Sciences Ethics Committee (no. 12-09). Informed consent was not required from the original patients because the study did not involve clinical interactions with the patients.

References


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