Increase in SCCmec type IV strains affects trends in antibiograms of meticillin-resistant Staphylococcus aureus at a tertiary-care hospital

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2Infection Control Committee, Tokyo Medical University Hachioji Medical Center, 1163 Tatemachi, Hachioji, Tokyo 193-0998, Japan
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INTRODUCTION

Meticillin-resistant Staphylococcus aureus (MRSA) is one of the major pathogens in hospital and community settings. In Japan, the isolation rate of MRSA in hospital settings is higher than that of other countries. Thus, the appropriate use of antimicrobial agents is strongly recommended (Diekema et al., 2001; Niki et al., 2011).

Recently, MRSA strains have been divided into two categories: healthcare-associated (HA)-MRSA and community-acquired (CA)-MRSA. HA-MRSA strains, which are mainly found in inpatients, commonly exhibit both high-level and multidrug resistance against β-lactams, macrolides and fluoroquinolones. However, CA-MRSA strains, which are mainly found in healthy individuals or outpatients, tend to be susceptible to a variety of non-β-lactam antimicrobial agents, although many have virulence genes (Takano et al., 2009). In particular, Panton–Valentine leukocidin (PVL), the toxin reported in cases of morbidity and mortality, has been known to be mainly distributed in CA-MRSA worldwide (Vandenesch et al., 2003; Deresinski, 2005; Tristan et al., 2007).

The mecA gene is located on a mobile genetic element called the staphylococcal cassette chromosome (SCC). SCCmec types are classified into types I–XI based on the structural differences of the mec complexes and the ccr
gene complexes (International Working Group on the Classification of Staphylococcal Cassette Chromosome Elements, 2009; Li et al., 2011; Shore et al., 2011). SCCmec types I–III are mainly found in HA-MRSA, whilst types IV and V are mainly found in CA-MRSA (Boye et al., 2007).

The prevalence of CA-MRSA strains in hospital settings is a serious worldwide problem (DeLeo et al., 2010; Chuang & Huang, 2013), although little is known about the distribution of CA-MRSA strains in Japanese hospitals. Furthermore, HA-MRSA strains with CA-MRSA features have tended to increase worldwide (Campanile et al., 2009; Nichol et al., 2011). Additionally, large-scale surveillance has shown that the antimicrobial resistance of both HA-MRSA and CA-MRSA strains has decreased in recent years (Campanile et al., 2009; Landrum et al., 2012). Many reports presenting results of MRSA isolated from several hospitals over a short period have been published; however, studies on the long-term surveillance for MRSA are extremely limited (Campanile et al., 2009; Nichol et al., 2011; Takata et al., 2012). As such, long-term surveillance targeting a single hospital would be more detailed and show an accurate transition of MRSA, because it is possible to more consistently investigate the same conditions. Therefore, the aim of this study was to investigate the annual transitions of MRSA strains with the CA-MRSA feature, which have been classified into SCCmec type IV or V, in a hospital setting in Japan. We investigated the annual transitions of the SCCmec types, rates of possession of virulence factors and antimicrobial susceptibilities for MRSA strains isolated from a tertiary-care hospital in Tokyo, Japan, between 2005 and 2012.

**METHODS**

**Bacterial strains.** During 2005–2012, 2698 isolates were initially screened as being MRSA in Tokyo Medical University, Hachioji

<table>
<thead>
<tr>
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<td>27 (1.2)</td>
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<td>12 (2.5)</td>
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<tr>
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<td>5 (0.8)</td>
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<td>0</td>
<td>1 (0.1)</td>
<td></td>
</tr>
<tr>
<td>Orthopaedic Surgery</td>
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<td>3 (0.5)</td>
<td>8 (1.6)</td>
<td>8 (1.5)</td>
<td>41 (1.8)</td>
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<tr>
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<td>57 (8.1)</td>
<td>18 (3.0)</td>
<td>61 (12.5)</td>
<td>20 (3.8)</td>
<td>156 (6.7)</td>
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<td>Paediatrics</td>
<td>59 (8.3)</td>
<td>13 (2.1)</td>
<td>41 (8.4)</td>
<td>20 (3.8)</td>
<td>133 (5.7)</td>
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</tr>
<tr>
<td>Plastic Surgery</td>
<td>17 (2.4)</td>
<td>5 (0.8)</td>
<td>12 (2.5)</td>
<td>22 (4.1)</td>
<td>56 (2.4)</td>
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<tr>
<td>Respiratory Medicine</td>
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<td>6 (1.0)</td>
<td>5 (1.0)</td>
<td>13 (2.4)</td>
<td>24 (1.0)</td>
<td></td>
</tr>
<tr>
<td>Respiratory Surgery</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (0.2)</td>
<td>1 (0.1)</td>
<td></td>
</tr>
<tr>
<td>Rheumatology</td>
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<td>0</td>
<td>0</td>
<td>2 (0.4)</td>
<td>2 (0.1)</td>
<td></td>
</tr>
<tr>
<td>Urology</td>
<td>18 (2.5)</td>
<td>7 (1.1)</td>
<td>24 (4.9)</td>
<td>9 (1.7)</td>
<td>58 (2.5)</td>
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<td>272 (38.4)</td>
<td>482 (79.0)</td>
<td>84 (17.2)</td>
<td>221 (41.5)</td>
<td>1059 (45.3)</td>
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</tr>
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</table>
SCC\textit{mec} types I–V were classified as non-typable (Boye et al., 2006) (Nakaminami et al., 2008). N315 (type II), 85/2082 (type III), JCSC 4744 (type IV) and WIS (type V) (Nakaminami et al., 2008).

**Growth condition and bacterial identification.** All strains were cultured on tryptone soy agar (Oxoid) under aerobic conditions at 35 °C for 24 h. All collected isolates were confirmed as \textit{S. aureus} by growth on mannitol salt agar (Oxoid), Gram staining and coagulase production (PS Latex; Eiken Chemical) (Nakaminami et al., 2008). Furthermore, MRSA strains were identified by coagulase testing, growth on Mueller-Hinton agar (Oxoid) containing 6 μg oxacillin ml\(^{-1}\) (Sigma-Aldrich) and 4% NaCl, and PCR-based detection of the \textit{mecA} gene (Noguchi et al., 2006).

**PCR amplification.** PCR assays for the detection of \textit{mecA}, the toxic shock syndrome toxin-1 gene \textit{tst} and the PVL gene \textit{pvl} were performed as described previously (Nakaminami et al., 2008). PCR was performed in the following cycles: for \textit{mecA}, 25 cycles (30 s denaturation at 95 °C, 30 s annealing at 58 °C and 30 s extension at 72 °C) and for \textit{tst} and \textit{pvl}, 25 cycles (30 s denaturation at 95 °C, 30 s annealing at 55 °C and 60 s extension at 72 °C). \textit{SCCmec} typing was performed according to the method of Boye et al. (2007), and the strains not identified as \textit{SCCmec} types I–V were classified as non-typable (Boye et al., 2007). PCR for \textit{SCCmec} typing was performed in the following cycles: 30 cycles (30 s denaturation at 94 °C, 30 s annealing at 55 °C and 60 s extension at 72 °C). The PCR samples were prepared in 100 μl sterile water containing a single MRSA colony, which was isolated from a streaking plate (Noguchi et al., 2006).

**Antimicrobial susceptibility testing.** MICs were determined by an agar doubling-dilution method, according to the Clinical and Laboratory Standards Institute guidelines (CLSI, 2009). The following antimicrobial agents were used: ampicillin (Wako), oxacillin (Sigma-Aldrich), cefotaxime (Wako), levofloxacin (Wako), sitafloxacin (Daichi Sankyo), clarithromycin (Wako), gentamicin (Wako), arbekacin (Meiji Seika Pharma), minocycline (Sigma-Aldrich), vancomycin (Sigma-Aldrich), teicoplanin (Sanofi) and linezolid (Pﬁzer). The breakpoints of these antimicrobial agents were determined using the Clinical and Laboratory Standards Institute interpretation criteria and unknown breakpoints were defined in this study (Nakaminami et al., 2014).

**Statistical analysis.** The annual transition of the MRSA strains was compared every 2 years. Differences in the rates of antimicrobial resistance, \textit{SCCmec} types and toxin genes were tested by a \(\chi^2\) test using JMP software (SAS Institute). \(P<0.05\) was considered statistically significant.

*\(P<0.05\) versus percentage of strains in 2011–2012, as determined by the \(\chi^2\) test.

### Table 2. Annual transition of \textit{SCCmec} types in MRSA strains between 2005 and 2012

<table>
<thead>
<tr>
<th>SCC\textit{mec} type</th>
<th>Strains ([n %(\text{n=708})])</th>
<th>Strains ([n %(\text{n=610})])</th>
<th>Strains ([n %(\text{n=488})])</th>
<th>Strains ([n %(\text{n=533})])</th>
<th>Total ([n %(\text{n=2339})])</th>
</tr>
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<tbody>
<tr>
<td>I</td>
<td>9 (1.3)</td>
<td>19 (3.1)</td>
<td>11 (2.3)</td>
<td>9 (1.7)</td>
<td>48 (2.1)</td>
</tr>
<tr>
<td>II</td>
<td>637 (90.0*)</td>
<td>537 (88.0*)</td>
<td>405 (83.0*)</td>
<td>396 (74.3)</td>
<td>1975 (84.4)</td>
</tr>
<tr>
<td>IV</td>
<td>41 (5.8*)</td>
<td>35 (5.7*)</td>
<td>51 (10.5*)</td>
<td>87 (16.3)</td>
<td>214 (9.1)</td>
</tr>
<tr>
<td>V</td>
<td>2 (0.3)</td>
<td>3 (0.5)</td>
<td>1 (0.2)</td>
<td>7 (1.3)</td>
<td>13 (0.6)</td>
</tr>
<tr>
<td>Non-typable</td>
<td>19 (2.7*)</td>
<td>16 (2.6*)</td>
<td>20 (4.1)</td>
<td>34 (6.4)</td>
<td>89 (3.8)</td>
</tr>
</tbody>
</table>

*\(P<0.05\) versus percentage of strains in 2011–2012, as determined by the \(\chi^2\) test.

### Table 3. Comparison between clinical departments and \textit{SCCmec} types in MRSA strains isolated in this study

<table>
<thead>
<tr>
<th>Department</th>
<th>SCC\textit{mec} type I/II ([n=2023])</th>
<th>SCC\textit{mec} type IV/V ([n=227])</th>
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</thead>
<tbody>
<tr>
<td>Cardiovascular Internal Medicine</td>
<td>42 (2.1)</td>
<td>1 (0.4)</td>
</tr>
<tr>
<td>Cardiovascular Surgery</td>
<td>67 (3.3)</td>
<td>5 (2.2)</td>
</tr>
<tr>
<td>Dermatology</td>
<td>87 (4.3)</td>
<td>27 (11.9*)</td>
</tr>
<tr>
<td>Diabetes, Endocrine and Metabolism Medicine</td>
<td>1 (0.1)</td>
<td>0</td>
</tr>
<tr>
<td>Emergency Medical Care Center</td>
<td>133 (6.6)</td>
<td>17 (7.5)</td>
</tr>
<tr>
<td>Gastroenterological Medicine</td>
<td>23 (1.1)</td>
<td>3 (1.3)</td>
</tr>
<tr>
<td>Gastroenterological Surgery and Transplantation</td>
<td>122 (6.0)</td>
<td>13 (5.7)</td>
</tr>
<tr>
<td>General Physics</td>
<td>0</td>
<td>1 (0.4)</td>
</tr>
<tr>
<td>Geriatrics</td>
<td>36 (1.8)</td>
<td>2 (0.9)</td>
</tr>
<tr>
<td>Glandula Mammaria</td>
<td>1 (0.1)</td>
<td>0</td>
</tr>
<tr>
<td>Haematology</td>
<td>20 (1.0)</td>
<td>0</td>
</tr>
<tr>
<td>Intensive Care Unit</td>
<td>29 (1.4)</td>
<td>1 (0.4)</td>
</tr>
<tr>
<td>Nephrology</td>
<td>44 (2.2)</td>
<td>10 (4.4)</td>
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<tr>
<td>Neurological Medicine</td>
<td>33 (1.6)</td>
<td>3 (1.3)</td>
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<td>Neurosurgery</td>
<td>36 (1.8)</td>
<td>2 (0.9)</td>
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<td>0</td>
</tr>
<tr>
<td>Orthopaedic Surgery</td>
<td>32 (1.6)</td>
<td>9 (4.0*)</td>
</tr>
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<td>Otolaryngology – Head and Neck Surgery</td>
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<tr>
<td>Paediatrics</td>
<td>101 (5.0)</td>
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<tr>
<td>Plastic Surgery</td>
<td>43 (2.1)</td>
<td>12 (5.3*)</td>
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<tr>
<td>Rheumatology</td>
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<tr>
<td>Urology</td>
<td>51 (2.5)</td>
<td>3 (1.3)</td>
</tr>
<tr>
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<td>954 (47.2)</td>
<td>68 (30.0*)</td>
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</table>

*\(P<0.05\) versus percentage of strains with SCC\textit{mec} type I/II, as determined by the \(\chi^2\) test.
that of type IV SCC despite the hospital setting. The MRSA strains were classified into SCC types I, V or non-typable: 48 (2.1%), 13 (0.6%) and 89 (3.8%) strains, respectively. No MRSA isolate was classified into SCC type III. The rate of detection of type II SCC was significantly decreased from 90.0 (2005–2006) to 74.3% (2011–2012) (P<0.01), whilst that of type IV SCC was significantly increased from 5.8 (2005–2006) to 16.3% (2011–2012) (P<0.01). When the clinical departments and SCCmeC types of MRSA strains were compared, the rates of detection of the strains carrying SCCmeC type IV or V were significantly higher than those of the strains carrying SCCmeC type I or II in the Departments of Dermatology, Orthopaedic Surgery, Paediatrics and Plastic Surgery (Table 3).

**Detection of virulence factors**

When the detection of toxin genes was conducted, *tst* and *pvl* genes were found in 1501 (64.2%) and 17 (0.7%) MRSA strains, respectively (Table 4). The rate of detection of the *tst* gene was significantly decreased from 66.7 (2005–2006) to 51.6% (2011–2012) (P<0.01), whilst that of the *pvl* gene was significantly increased from 0.1 (2005–2006) to 2.1% (2011–2012) (P<0.01). In the strains carrying the *tst* gene, 0.3, 89.1, 5.7, 0.1 and 4.7% were SCCmeC

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**Table 4. Distribution of *tst* and *pvl* genes in MRSA strains isolated in this study**

<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td><em>tst</em></td>
<td>472 (66.7*)</td>
<td>432 (70.8*)</td>
<td>322 (66.0*)</td>
<td>275 (51.6)</td>
<td>1501 (64.2)</td>
</tr>
<tr>
<td><em>pvl</em></td>
<td>1 (0.1*)</td>
<td>5 (0.8)</td>
<td>0</td>
<td>11 (2.1)</td>
<td>17 (0.7)</td>
</tr>
</tbody>
</table>

*P<0.05 versus percentage of strains in 2011–2012, as determined by the χ² test.

**RESULTS**

**SCCmeC typing**

After confirmation in our laboratory, a total of 2339 isolates were identified as being MRSA (Table 1). SCCmeC types of these isolates are shown in Table 2.

In the present study, type II SCCmeC, which is mainly found in HA-MRSA, was detected in 1975 (84.4%) MRSA strains. Type IV SCCmeC, which is mainly found in CA-MRSA, was detected in 214 (9.2%) MRSA strains despite the hospital setting. The MRSA strains were classified into SCCmeC types I, V or non-typable: 48 (2.1%), 13 (0.6%) and 89 (3.8%) strains, respectively. No MRSA isolate was classified into SCCmeC type III. The rate of detection of type II SCCmeC was significantly decreased from 90.0 (2005–2006) to 74.3% (2011–2012) (P<0.01), whilst that of type IV SCCmeC was significantly increased from 5.8 (2005–2006) to 16.3% (2011–2012) (P<0.01). When the clinical departments and SCCmeC types of MRSA strains were compared, the rates of detection of the strains carrying SCCmeC type IV or V were significantly higher than those of the strains carrying SCCmeC type I or II in the Departments of Dermatology, Orthopaedic Surgery, Paediatrics and Plastic Surgery (Table 3).

**Detection of virulence factors**

When the detection of toxin genes was conducted, *tst* and *pvl* genes were found in 1501 (64.2%) and 17 (0.7%) MRSA strains, respectively (Table 4). The rate of detection of the *tst* gene was significantly decreased from 66.7 (2005–2006) to 51.6% (2011–2012) (P<0.01), whilst that of the *pvl* gene was significantly increased from 0.1 (2005–2006) to 2.1% (2011–2012) (P<0.01). In the strains carrying the *tst* gene, 0.3, 89.1, 5.7, 0.1 and 4.7% were SCCmeC

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**Table 5. Antimicrobial susceptibilities of MRSA strains isolated in this study**

<table>
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</thead>
<tbody>
<tr>
<td><strong>MIC</strong>(<em>50/90</em>)(µg ml⁻¹)</td>
<td><strong>R (%)</strong></td>
<td><strong>MIC</strong>(<em>50/90</em>)(µg ml⁻¹)</td>
<td><strong>R (%)</strong></td>
<td><strong>MIC</strong>(<em>50/90</em>)(µg ml⁻¹)</td>
<td><strong>R (%)</strong></td>
</tr>
<tr>
<td>Ampicillin</td>
<td>32/64</td>
<td>99.9</td>
<td>32/64</td>
<td>100</td>
<td>32/64</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>≥128/128</td>
<td>96.8*</td>
<td>≥128/128</td>
<td>94.6*</td>
<td>≥128/128</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>32/128</td>
<td>92.2*</td>
<td>32/128</td>
<td>92.1*</td>
<td>16/≥128</td>
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<tr>
<td>Sulfamethoxazole</td>
<td>1/16</td>
<td>50.0</td>
<td>2/32</td>
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<td>1/32</td>
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<td>Clarithromycin</td>
<td>≥128/128</td>
<td>95.6*</td>
<td>≥128/128</td>
<td>95.2*</td>
<td>≥128/128</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>32/128</td>
<td>64.7</td>
<td>64/≥128</td>
<td>65.4</td>
<td>64/≥128</td>
</tr>
<tr>
<td>Arbekacin</td>
<td>0.25/1</td>
<td>0.6</td>
<td>0</td>
<td>0.5/1</td>
<td>0.8</td>
</tr>
<tr>
<td>Minocycline</td>
<td>32/32</td>
<td>73.2*</td>
<td>16/32</td>
<td>63.6*</td>
<td>8/32</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>1/1</td>
<td>0</td>
<td>1/1</td>
<td>0</td>
<td>1/1</td>
</tr>
<tr>
<td>Teicoplanin†</td>
<td>ND</td>
<td>ND</td>
<td>1/2</td>
<td>0</td>
<td>1/2</td>
</tr>
<tr>
<td>Linezolid‡</td>
<td>1/2</td>
<td>0</td>
<td>1/2</td>
<td>0</td>
<td>1/2</td>
</tr>
</tbody>
</table>

*P<0.05 versus the antimicrobial susceptibilities in 2011–2012, as determined by the χ² test.

†Data were available from 2008 (n=283).
‡Data were available from 2006 (n=340).
type I, II, IV, V and non-typable, respectively. However, 17 *pvl*-positive strains were divided into 11.8, 82.4 and 5.9% SCCmec type II, IV and non-typable, respectively. Interestingly, two strains carrying both *tsr* and *pvl* genes were detected and classified into SCCmec type IV.

**Antimicrobial susceptibility**

The antimicrobial susceptibilities of all MRSA strains were determined (Table 5). As no significant difference was found in the MIC distributions, no notable change was observed in the MIC<sub>50</sub> and MIC<sub>90</sub> values of the antimicrobial agents, excluding minocycline. Although no notable transition of the resistance rates of penicillins was observed, the resistance rate of cefotaxime was significantly decreased every year (*P*<0.05). The resistance rates of fluoroquinolones showed a tendency to decrease. In addition, the resistance rate of clarithromycin was significantly decreased every year (*P*<0.01). No notable transition of the resistance rate of gentamicin was observed. The resistance rate of minocycline was significantly decreased every year (*P*<0.01). All strains were susceptible to vancomycin, teicoplanin and linezolid. Sixteen isolates were resistant (MIC ≥ 8 µg ml<sup>−1</sup>) to arbekacin.

**Antimicrobial susceptibilities in SCCmec type I/II and IV/V strains**

The antimicrobial susceptibilities for the strains carrying SCCmec types I/II and IV/V were compared (Table 6). The MIC<sub>50</sub>s of β-lactams, fluoroquinolones, clarithromycin, gentamicin and minocycline for the strains carrying SCCmec type IV/V were lower than those for the strains carrying SCCmec type I/II. For ampicillin, sitafloxacin, arbekacin, minocycline and teicoplanin, MIC<sub>50</sub>s for the strains carrying SCCmec type IV/V were lower than those for the strains carrying SCCmec type I/II. Although no significant differences were found in the resistance rates of penicillins, the resistance rates of cefotaxime, levofloxacin, sitafloxacin, clarithromycin and minocycline for the strains carrying SCCmec type IV/V were significantly lower than those for the strains carrying SCCmec type I/II (*P*<0.01, respectively). In contrast, the resistance rate of gentamicin for the strains carrying SCCmec type IV/V was higher than that for the strains carrying SCCmec type I/II. No notable differences of the susceptibility to anti-MRSA agents were observed.

**DISCUSSION**

Long-term surveillance of MRSA strains isolated from the hospital setting provides a way to predict the annual transitions of virulence factors and antibiograms. The information will contribute to the effective control of hospital infections and the treatment of infections caused by MRSA.

In this study, MRSA strains of SCCmec type II, which are mainly found in HA-MRSA, were significantly decreased, whilst those of SCCmec type IV, which are mainly found in CA-MRSA, were significantly increased, as also seen in other countries (Nichol et al., 2011). Many strains carrying SCCmec type I/II were isolated from patients undergoing long-term hospitalization, such as in the Department of Surgery. In contrast, the strains of SCCmec type IV/V were frequently isolated from patients of the Departments of Dermatology and Paediatrics, which contain many outpatients. Therefore, the MRSA strains with CA-MRSA features isolated in the hospital setting were presumed to be transmitted from the community setting via outpatients. It will be necessary to note the trend of CA-MRSA in the hospital setting in the future.

With regard to the annual transition of virulence factors, the rate of detection of the *tsr* gene was found to significantly decrease every year and 89.1% of the strains carrying *tsr* were SCCmec type II, indicating that the trend of decreasing numbers of strains carrying *tsr* is presumed to be related to a decrease in the strains carrying SCCmec type II. However, the ratio of the *pvl*-positive strains was found to increase every year and the *pvl* gene was mainly found in the strains of SCCmec type IV in this study.

**Table 6. Comparison of antimicrobial susceptibilities between the strains of SCCmec types I/II and IV/V**

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>SCCmec type I/II (n=2023)</th>
<th>SCCmec type IV/V (n=227)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC&lt;sub&gt;50&lt;/sub&gt;/MIC&lt;sub&gt;90&lt;/sub&gt; (µg ml&lt;sup&gt;−1&lt;/sup&gt;)</td>
<td>R (%)</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>32/64 100</td>
<td>16/32 100</td>
</tr>
<tr>
<td>Oxacillin</td>
<td>≥128/≥128 99.8</td>
<td>64/≥128 99.6</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>≥128/≥128 96.5</td>
<td>64/≥128 59.9&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>32/≥128 94.4</td>
<td>8/≥128 55.5&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sitafloxacin</td>
<td>1/16 47.7</td>
<td>0.5/8 29.1&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>Clarithromycin</td>
<td>≥128/≥128 97.3</td>
<td>16/≥128 58.1&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>64/≥128 64.5</td>
<td>32/≥128 67.0</td>
</tr>
<tr>
<td>Arbekacin</td>
<td>0.5/2 0.5</td>
<td>0.5/1 1.8</td>
</tr>
<tr>
<td>Minocycline</td>
<td>16/32 66.8</td>
<td>0.13/16 13.7&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>1/1 0</td>
<td>1/1 0</td>
</tr>
<tr>
<td>Teicoplanin†</td>
<td>1/2 0</td>
<td>1/1 0</td>
</tr>
<tr>
<td>Linezolid‡</td>
<td>1/2 0</td>
<td>1/2 0</td>
</tr>
</tbody>
</table>

<sup>*</sup>P<0.05 versus the antimicrobial susceptibilities of MRSA with SCCmec type I/II, as determined by the χ<sup>2</sup> test. †n=1077 (SCCmec type I/II) and 168 (SCCmec type IV/V). ‡n=1688 (SCCmec type I/II) and 208 (SCCmec type IV/V).
In the US and Europe, the \textit{pvl} gene has been known to be mainly distributed in CA-MRSA strains (Zetola \textit{et al.}, 2005). However, it was reported that PVL-positive MRSA infections have been rare in Japan since 2000 (Isobe \textit{et al.}, 2012). Our results indicate CA-MRSA carrying \textit{pvl} may be gradually increasing in Japan.

With regard to antimicrobial susceptibility testing, whilst no notable differences of MIC distribution were observed, the resistance rates of antimicrobial agents, excluding ampicillin, oxacillin, gentamicin, and arbekacin, showed a decreasing trend. These results suggest that the increase in the number of strains exhibiting an under-breakpoint of the MIC led to a decrease in the resistance rates of the antimicrobial agents. Additionally, the resistance rates of cefotaxime, levofloxacin, sitafloxacin, clarithromycin and minocycline for the strains carrying SCC\textit{mec} type IV/V were significantly lower than those for the strains carrying SCC\textit{mec} type I/II. Generally, HA-MRSA strains exhibit high-level and multidrug resistance against non-\textit{\beta}-lactam antimicrobial agents as compared with CA-MRSA strains. As the strains with SCC\textit{mec} type I/II accounted for ~90% of all strains in 2005–2006, it can be presumed that the annual transitions of antimicrobial susceptibility to cefotaxime, levofloxacin, clarithromycin and minocycline were affected by the decreased prevalence of the type I/II cassette in later years. In contrast, the resistance rate of gentamicin for the strains carrying SCC\textit{mec} type IV/V was higher than that for the strains carrying SCC\textit{mec} type I/II. In this study, 16 arbekacin-resistant strains were found. The details of the arbekacin-resistant strains are still unknown. Further study is necessary to understand these arbekacin-resistant strains.

Thus, our results show that strains with CA-MRSA features invaded the hospital setting, contributing to the change of antimicrobial agent susceptibility and the prevalence of toxin genes in MRSA isolated from the hospital. In the future, it can be presumed that the strains with CA-MRSA features, which indicate susceptibility to antimicrobial agents and high toxicity, will increase in hospitals and infection control may be considered in the community.

**ACKNOWLEDGEMENTS**

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