Comparative prevalence of superantigenic toxin genes in meticillin-resistant and meticillin-susceptible *Staphylococcus aureus* isolates

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A total of 118 meticillin-resistant *Staphylococcus aureus* (MRSA) and 140 meticillin-susceptible *S. aureus* (MSSA) isolates from different patients in the same time period were comprehensively searched using a multiplex PCR for the classical and recently described superantigenic toxin gene family comprising the staphylococcal enterotoxin genes *sea* to *ser* and the toxic shock syndrome toxin 1 gene, *tst-1*. Both MRSA and MSSA isolates carried a number of superantigenic toxin genes, but the MRSA isolates harboured more superantigenic toxin genes than the MSSA isolates. The most frequent genotype of the MRSA isolates was *sec, sell* and *tst-1* together with the gene combination *seg, sei, selm, seln* and *selo*, which was found strictly in combination in 69.5% of the isolates tested. In contrast, possession of the *sec, sell* and *tst-1* genes in MSSA isolates was significantly less than in MRSA (2.1 vs 77.1%, respectively), although they also often contained the combination genes (25.0%). This notable higher prevalence in MRSA isolates indicated that possession of the *sec, sell* and *tst-1* genes in particular appeared to be a habitual feature of MRSA. Moreover, these were mainly due to the fixed combinations of the mobile genetic elements type I *vsA4* encoding *sec, sell* and *tst-1*, and type I *vsAβ* encoding *seg, sei, selm, seln* and *selo*. Analysis of the relationship between toxin genotypes and the toxin gene-encoding profiles of mobile genetic elements has a possible role in determining superantigenic toxin genotypes in *S. aureus*.

INTRODUCTION

*Staphylococcus aureus* is a leading cause of human disease in the hospital setting, as well as in the community, accounting for a wide range of diseases from superficial skin infections to life-threatening conditions, such as bacteraemia, endocarditis, pneumonia and toxic shock syndrome (Hallin et al., 2007; Lowy, 1998). Infections with *S. aureus* are especially difficult to treat because of evolved resistance to antimicrobial drugs. The emergence of meticillin-resistant *S. aureus* (MRSA) strains and other antimicrobial agents has become a major concern, especially in the hospital environment, because of increased mortality due to systemic MRSA infections (Klein et al., 2007). Meticillin resistance is conferred by carriage of the *mecA* gene (Beck et al., 1986; Katayama et al., 2000), which is carried by a mobile exogenous genetic element known as the staphylococcal cassette chromosome *mec* (SCCmec) (Archer et al., 1996; Wu et al., 1996). Analysis of the natural population dynamics and expansion of pathogenic clones of *S. aureus* has provided evidence that essentially any *S. aureus* genotype carried by humans can transform into a life-threatening human pathogen, but that certain clones are more virulent than others (Melles et al., 2004).

Many *S. aureus* strains, especially MRSA, produce one or more specific staphylococcal exotoxins, including staphylococcal enterotoxins (SEs), enterotoxin-like superantigens and toxic shock syndrome toxin 1 (TSST-1). These toxins have been classified as members of the pyrogenic toxin superantigenic toxin gene family comprising the staphylococcal enterotoxin genes *sea* to *ser* and the toxic shock syndrome toxin 1 gene, *tst-1*.
superantigen family because of their biological activities and structural relatedness. Superantigens bypass normal antigen presentation and have strong T-cell mitogenic activity as a result of direct binding to the Vβ region of specific T cells and the major histocompatibility complex class II molecules of antigen-presenting cells (Llewelyn & Cohen, 2002). This leads to a massive release of pro-inflammatory cytokines, such as tumour necrosis factor, interleukin-2, interleukin-6 and gamma interferon, which is responsible for the physiopathology of toxic shock syndrome (Holtfreter & Broker, 2005; McCormick et al., 2001) and contributes to the severity of S. aureus sepsis (Holtfreter & Broker, 2005). SEs were originally divided into five serological types (SEA–SEE) based on their antigenicity. In recent years, new types of SE and SE-like toxins (SEG, SEH, SEI, SEII, SEIK, SEIL, SEIM, SEIN, SEIO, SEIP, SEIQ, SEIR and SEIU) have been reported (Jarraud et al., 2001; Letertre et al., 2003; McCormick et al., 2001; Munson et al., 1998; Omoe et al., 2003, 2004; Su & Wong, 1995). Little information is available about the degree of superantigen genetic variability among populations of clinical meticillin-susceptible S. aureus (MSSA) and community S. aureus. The latter is a population in which selection pressure for virulence and resistance may not occur. This raises the question of whether community and MSSA isolates resemble MRSA isolates in their clonal structure, and whether their genomes are conserved to the extent seen in MRSA populations. An answer to this question may provide a better understanding of the capacity of this bacterium to cause infection or remain commensal. Recently, we described a comprehensive detection system for 18 types of classical and newly described staphylococcal superantigenic toxin genes using four sets of multiplex PCR (Omoe et al., 2005). The purpose of the present study was to investigate the presence of the staphylococcal superantigen toxin genes in sets of MSSA and MRSA isolates collected from different patients during 2004–2007, and to correlate them with toxin gene profiles.

METHODS

**Bacteria isolates and culture conditions.** A total of 258 S. aureus isolates from clinical specimens was collected from different patients admitted to Hirosaki University Hospital, Japan, during 2004–2007 and used in this study (Table 1). Of these, 56 strains were isolated from expectoration, 42 from cellulitis and abscess, and 39 from pharynx viscous liquid, whilst other strains were isolated from faeces and urine (27 strains), blood (20 strains), absorption slime in the lung (18 strains), cannulas (18 strains), discharge from the ear (16 strains), wounds (15 strains) and the nasal cavity (7 strains). Each sample was plated onto trypticase soy agar with 5% defibrinated sheep blood (Nissui Pharmaceutical) and incubated at 35°C for 24 h. S. aureus was identified by standard microbiological methods, including Gram staining, a catalase test, a latex slide agglutination test for clumping factor and protein A (PS Test; Eiken Chemistry) and a tube coagulase test. The strains were stored in trypticase soy broth with 15% glycerol at -85°C until use. Eleven reference strains including full-genome sequenced strains (N315, GenBank accession no. BA000018; Mu50, GenBank accession BA000017; MW2, GenBank accession BA000033) were used as reference strains in this study (Omoe et al., 2005).

<table>
<thead>
<tr>
<th>Source of clinical strain</th>
<th>No. of patients with MRSA (%) (n=118)</th>
<th>No. of patients with MSSA (%) (n=140)</th>
<th>Total (n=258)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hospital ward/department</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surgery</td>
<td>46 (38.1)</td>
<td>28 (20.0)</td>
<td>74</td>
</tr>
<tr>
<td>Internal medicine</td>
<td>18 (15.3)</td>
<td>20 (14.3)</td>
<td>38</td>
</tr>
<tr>
<td>Paediatrics</td>
<td>16 (13.6)</td>
<td>45 (32.1)</td>
<td>61</td>
</tr>
<tr>
<td>Urology</td>
<td>7 (5.9)</td>
<td>2 (1.4)</td>
<td>9</td>
</tr>
<tr>
<td>Dermatology</td>
<td>13 (11.0)</td>
<td>8 (5.7)</td>
<td>21</td>
</tr>
<tr>
<td>Obstetrics and gynaecology</td>
<td>2 (1.7)</td>
<td>8 (5.7)</td>
<td>10</td>
</tr>
<tr>
<td>Otolaryngology</td>
<td>13 (11.0)</td>
<td>20 (14.3)</td>
<td>33</td>
</tr>
<tr>
<td>Other</td>
<td>3 (2.5)</td>
<td>9 (6.4)</td>
<td>12</td>
</tr>
<tr>
<td><strong>Source/body site</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cannula and drain</td>
<td>7 (5.9)</td>
<td>11 (7.9)</td>
<td>18</td>
</tr>
<tr>
<td>Pharynx viscous liquid</td>
<td>14 (11.9)</td>
<td>25 (17.9)</td>
<td>39</td>
</tr>
<tr>
<td>Expectoration</td>
<td>33 (28.0)</td>
<td>23 (16.4)</td>
<td>56</td>
</tr>
<tr>
<td>Nasal cavity</td>
<td>2 (1.7)</td>
<td>5 (3.6)</td>
<td>7</td>
</tr>
<tr>
<td>Absorption slime in lung</td>
<td>3 (2.5)</td>
<td>15 (10.7)</td>
<td>18</td>
</tr>
<tr>
<td>Discharge from ear</td>
<td>9 (7.6)</td>
<td>7 (5.0)</td>
<td>16</td>
</tr>
<tr>
<td>Blood</td>
<td>13 (11.0)</td>
<td>7 (5.0)</td>
<td>20</td>
</tr>
<tr>
<td>Wound</td>
<td>6 (5.1)</td>
<td>9 (6.4)</td>
<td>15</td>
</tr>
<tr>
<td>Cellulitis and abscess</td>
<td>22 (18.6)</td>
<td>20 (14.3)</td>
<td>42</td>
</tr>
<tr>
<td>Faeces and urine</td>
<td>9 (7.6)</td>
<td>18 (12.9)</td>
<td>27</td>
</tr>
</tbody>
</table>
Antimicrobial susceptibility testing. Antimicrobial susceptibility testing was performed with the PoS Combo type 2J panel and the results read on a WalkAway system (Dade Behring). The antibiotics tested were gentamicin, erythromycin, clindamycin, ofloxacin, vancomycin and trimethoprim/sulfamethoxazole. Isolates were interpreted as sensitive or resistant according to their MICs, as described by the NCCLS (2001). Resistance to several drugs was determined by plating on trypticase soy agar containing antibiotics. After 24 h incubation, growth of more than two colonies was determined as resistance. In addition, resistance to meticillin was detected on oxacillin resistance screening agar (Mueller–Hinton agar + oxacillin) and was confirmed by screening for penicillin-binding protein 2a (SlideX MRSA detection; Denka Seiken) (Murakami et al., 1991).

DNA purification and primers. Total DNA from S. aureus was purified with a QIAamp DNA purification kit (Qiagen) according to the manufacturer’s instructions. The concentration of DNA was determined according to A260 values. The nucleotide sequences of all PCR primers and their respective amplified products have been described previously (Omoe et al., 2005). The primer sets were designed to anneal to unique regions and to generate amplicons that would allow identification of each se or tst gene based on the molecular mass of its PCR product. As an internal positive control, products were resolved by electrophoresis in a 3 % NuSieve 3:1 agarose gel, followed by 35 cycles of 95°C for 1 min, denaturation of DNA and Taq polymerase activation at 95°C for 15 min, followed by 35 cycles of 95°C for 30 s, 57°C for 90 s and 72°C for 90 s, followed by a final extension at 72°C for 10 min. PCR products were resolved by electrophoresis in a 3 % NuSieve 3:1 agarose gel (Cambrex Bio Science) in 0.5× Tris/boric acid/EDTA buffer, stained with 0.5 µg ethidium bromide ml⁻¹ and visualized on a transilluminator.

Statistical analysis. The prevalence of the tested genes in the MRSA and MSSA isolates was compared using a two-tailed Fisher’s exact test. A significance level of 0.05 was used.

RESULTS

Distribution of MRSA and MSSA isolates in different hospital departments and body sites

In total, 118 consecutive MRSA and 140 MSSA strains isolated from clinical specimens were studied (Table 1). The highest proportions of MRSA clones were isolated from the surgical wards (38.1 %) and the Department of Internal Medicine (15.3 %). In contrast, the highest proportions of MSSA clones were isolated from paediatric wards (32.1 %), and the Departments of Internal Medicine (14.3 %) and Otolaryngology (14.3 %). The patients with MRSA infection or colonization were older than the patients with MSSA infection. The distribution of MRSA and MSSA among the various body sites was similar among the patient groups infected with MRSA or MSSA (Table 1).

Prevalence of superantigenic toxin genes in MRSA and MSSA isolates

We recently developed a comprehensive detection system for 18 types of classical and newly described staphylococcal superantigenic toxin genes using four sets of multiplex PCRs (Omoe et al., 2005). In the present study, a total of 140 MSSA isolates and 118 MRSA isolates from clinical specimens was subjected to genotyping analysis of superantigenic toxin genes. All of the MRSA and MSSA isolates tested harboured the femA and femB genes. A total of 44 superantigenic toxin genotypes were detected among the 258 MRSA and MSSA isolates (Table 2). Of the 118 MRSA isolates, all strains were diagnosed as positive for superantigenic toxin genes. A total of 23 toxin genotypes was observed among the MRSA isolates, and the toxin genotype with the highest incidence (44.1 %, 52/118 strains) was sec, seg, sei, sell, selm, seln, selo, tst-1 (Table 2). In contrast to the MRSA isolates, 34 isolates (24.3 %) of the 140 MSSA isolates were diagnosed as negative for superantigenic toxin genes. A total of 26 toxin genotypes was observed among the MSSA isolates. The toxin genotype with the highest incidence (12.9 %, 18/140 strains) was seg, sei, selm, seln, selo, which is a combination of recently identified superantigenic toxin genes (Table 2).

Comparison of toxin genotypes in MRSA and MSSA isolates

All of the MRSA isolates included in the present study were positive for at least one of the superantigenic genes tested. All of the MRSA isolates harboured more than one superantigenic gene except for three strains (2.5 %), which contained selp only (Table 2). Of the MRSA isolates, 44.1 and 17.8 % contained eight and nine superantigenic genes, respectively (Table 2, Fig. 1). Twelve strains (10.2 %) contained 10 or 11 superantigenic genes. This genotype was mainly due to the fixed combination of seg, sei, selm, seln and selo genes. In contrast, for the MSSA isolates, 24.3 % (34 strains) were diagnosed as negative for superantigenic genes. Twenty of the gene-positive strains from the MSSA isolates contained only a single gene (14.3 % of all MSSA isolates). Interestingly, most of the MSSA isolates contained five or fewer superantigenic genes, although seven (5.0 %) strains contained eight genes (Fig. 1). These results indicated that MRSA isolates harboured more superantigenic genes than MSSA isolates.

We then compared the genotypes and frequency of superantigenic toxin genes in the MRSA and MSSA isolates. All superantigenic genes tested in this study except for the see gene were detected in the MRSA and MSSA isolates (Table 2, Fig. 2). Of the 118 MRSA isolates, 69.5 % of the isolates contained sec, sell, tst-1 and the gene combination seg, sei, selm, seln and selo. Of the 140 MSSA
Table 2. Prevalence of *S. aureus* se genotypes and relationship with mobile genetic elements

<table>
<thead>
<tr>
<th><em>S. aureus</em> se genotype</th>
<th>Prevalence</th>
<th>Suspected genomic island and plasmid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MRSA (n=118)</td>
<td>MSSA (n=140)</td>
</tr>
<tr>
<td>sea</td>
<td>–</td>
<td>7</td>
</tr>
<tr>
<td>sea, seg</td>
<td>–</td>
<td>1</td>
</tr>
<tr>
<td>sea, sec, sell</td>
<td>–</td>
<td>1</td>
</tr>
<tr>
<td>sea, sec, selo</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>sea, seb, selk, selq</td>
<td>–</td>
<td>1</td>
</tr>
<tr>
<td>sea, sec, selo, sell</td>
<td>2</td>
<td>–</td>
</tr>
<tr>
<td>sea, seg, sei, tst-1</td>
<td>–</td>
<td>3</td>
</tr>
<tr>
<td>sea, seh, selk, selq</td>
<td>–</td>
<td>11</td>
</tr>
<tr>
<td>sea, seg, sei, seln, tst-1</td>
<td>–</td>
<td>7</td>
</tr>
<tr>
<td>sea, seg, sei, seln, selo</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>sea, sec, seg, sell, seln, selo, tst-1</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>sea, sec, seg,sell, seln, seln, selo, selo, tst-1</td>
<td>8</td>
<td>–</td>
</tr>
<tr>
<td>sea, sec, seg, sei, sell, seln, seln, selo, selp, tst-1</td>
<td>10</td>
<td>–</td>
</tr>
<tr>
<td>seb</td>
<td>–</td>
<td>1</td>
</tr>
<tr>
<td>seb, selp</td>
<td>–</td>
<td>4</td>
</tr>
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<tr>
<td>sec, sell</td>
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<td>sec, seg, sei, selo</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>sec, seb, selp, tst-1</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>sec, seg, sei, sell, seln, selo, selo</td>
<td>2</td>
<td>–</td>
</tr>
<tr>
<td>sec, seg, sell, seln, selo, tst-1</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>sec, seg, sei, sell, seln, seln, selo, selo, selo, selp, tst-1</td>
<td>4</td>
<td>–</td>
</tr>
<tr>
<td>sec, sei, sell, seln, selo, selp, tst-1</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>sec, seg, sei, sell, seln, seln, selo, selo, tst-1</td>
<td>52</td>
<td>3</td>
</tr>
<tr>
<td>sec, seg, sei, sell, seln, seln, selo, selo, selp, tst-1</td>
<td>10</td>
<td>–</td>
</tr>
<tr>
<td>sec, sed, seg, sei, selj, sell, seln, seln, selo, selp, tst-1</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>sed, seg, sei, selj, selm, seln, selo, selr, tst-1</td>
<td>–</td>
<td>4</td>
</tr>
<tr>
<td>sed, selj, selr</td>
<td>2</td>
<td>–</td>
</tr>
<tr>
<td>seg, sei</td>
<td>–</td>
<td>1</td>
</tr>
<tr>
<td>seg, sei, seln</td>
<td>–</td>
<td>1</td>
</tr>
<tr>
<td>seg, sei, selo</td>
<td>–</td>
<td>1</td>
</tr>
<tr>
<td>seg, sei, tst-1</td>
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</tr>
<tr>
<td>seg, sei, selm, seln</td>
<td>–</td>
<td>10</td>
</tr>
<tr>
<td>seg, sei, seln, selo</td>
<td>7</td>
<td>18</td>
</tr>
<tr>
<td>seg, sei, selm, seln, selo, selp</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>selj, selr</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>selm, selo</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>selo</td>
<td>–</td>
<td>2</td>
</tr>
<tr>
<td>selp</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>Negative for se genes</td>
<td>–</td>
<td>34</td>
</tr>
</tbody>
</table>

isolates, although the combination genes (44.2% *seg*, 44.2% *sei*, 34.3% *selm*, 40.0% *seln*, 28.6% *selo*) were observed more frequently in the these isolates, just 10–12% of the isolates contained the *sec* (12.1%), *sell* (12.1%) or *tst-1* (10.0%) gene (Fig. 2).

### Analysis of superantigenic toxin gene-encoding mobile genetic elements in MRSA and MSSA isolates

It is known that almost all superantigenic toxin genes are associated with mobile genetic elements such as pathogenicity...
islands, prophages, SCCmec and plasmids. Thus, we analysed the relationship between superantigenic toxin genotypes obtained in this study and known superantigenic toxin-encoding mobile genetic elements. Twenty of the forty-four superantigenic toxin genotypes (45.5%) could be considered as combinations of the superantigenic toxin gene-encoding profiles of genomic islands and/or plasmids (Table 2). Most of the genetic elements observed in the MRSA isolates were a combination of type I vSaβ (83.1%), encoding seg, sei, selm, seln and selo, and type I vSa4 (77.1%), encoding sec, sell and tst-1 (Fig. 3). In the MSSA isolates, most of the mobile genetic elements were type I vSaβ (25.0%), but this was significantly lower than in the MRSA isolates. Type I vSa4 was just 2.1% of all the MSSA isolates.

DISCUSSION

The present study is, to our knowledge, the first cross-sectional and comprehensive comparison of the classical and newly described superantigenic toxin genotypes of MRSA and MSSA isolates by multiplex PCR. Our study showed that there are many superantigenic toxin genotypes in both MRSA and MSSA isolates from clinical specimens, but that the MRSA isolates harboured more superantigenic toxin genes than the MSSA isolates. Of the MRSA isolates, 68.5% had sec, sell, tst-1 and the combination genes (seg, sei, selm, seln and selo). However, only 10–12% of MSSA strains contained sec, sell or tst-1, although they also often contained the combination genes (25.0%). The genes sec, sell and tst-1 are associated with the mobile genetic element type I vSa4, whilst the combination genes (seg, sei, selm, seln and selo) are encoded by type I vSaβ, an enterotoxin gene cluster locus (egc).

MRSA is produced when MSSA acquires a mobile genetic element, SCCmec. Toxin-producing MSSA may also alter the pathogenicity of established MRSA by the transfer of virulence factors via plasmids or mobile elements. Characterizing the clinically relevant MRSA and testing for classical and newly described SEs, SE-like superantigens and TSST-1 provides additional information for epidemiological data analysis. It has generally been accepted that only some strains produce superantigenic toxins (Becker et al., 2003; Dinges et al., 2000). However, considering both the newly published se genes (seg to selr) and the classical toxins, the overall rate of toxin gene-positive isolates in the study presented here reached 86.8%. Recently, Chini et al. (2006) have shown that 60/177 MRSA isolates were positive for at least one of the superantigen genes. Omoe et al. (2005) also reported that all strains originating from food poisoning were positive for se genes, and 79.4% of isolates from healthy human nasal swabs were positive for se and/or tst genes. All of the MRSA and 75.7% of the MSSA isolates tested carried a number of toxin genes, ranging from 1 to 11, with extensive variation between individual strains. SEs, SE-like superantigens and TSST-1, which belong to the pyrogenic toxin superantigen family, are considered to be major virulence factors of S. aureus (Dinges et al., 2000; Lina et al., 2004). Most of the diseases induced by S. aureus are caused by specific toxins whose genetic determinants are frequently carried by potentially mobile elements such as plasmids (e.g. ets, sed), phages.
(e.g. sea, sep) or pathogenicity islands (e.g. tst, sec, sel, egc) (Jarraud et al., 2002; Kuroda et al., 2001). Expression of virulence genes in S. aureus is controlled by the accessory gene regulator (agr), with a polymorphism in the sequence of the autoinducing peptide and its receptor (Jarraud et al., 2002; Lina et al., 2003). Distribution of toxin genes is closely linked to the strain’s genetic background. Baba et al. (2002) suggested that genomic island allototyping would be a useful approach to S. aureus genotyping and that this process would enable the prediction of the pathogenic capability of S. aureus clinical strains. The present study has shown that our multiplex PCR system for comprehensive detection of superantigenic toxin genes would be useful for determining genomic island allotypes.

Interestingly, our results showed that 69.5% of the MRSA, but not the MSSA, isolates harboured sec, sell, tst-1 and the combination genes (seg, sei, selm, seln, selo) or had further combinations with other toxin genes. These data suggest that these MRSA isolates mainly are the New York/Japan clone, with multilocus sequence type 5, agr type 2 and SCCmec II, and possessing both the tst and sec genes (Zaraket et al., 2007). Furthermore, our results showed that the combination genes (seg, sei, selm, seln and selo) encoded by egc were incomplete in a few strains of both MRSA and MSSA, suggesting that the egc locus was incomplete and occasionally harboured an insertion sequence and transposable genes. These strains may represent evolutionary intermediates of the egc locus (Thomas et al., 2006). In general, bacteraemia isolates of S. aureus often contain the classical members of the superantigen family, isolates from patients with diarrhoea carry seh and isolates from wound infections harbour the sec gene (Uchiyama et al., 1994). In addition to SEs, TSST-1 of S. aureus is associated with septic shock and toxic shock syndrome (Uchiyama et al., 1989). The role of SEs, especially the recently described SEs, in toxin-mediated staphylococcal diseases and in increasing acquisition of resistance against antibiotics remains unclear. In view of our results and recently published studies, this should be investigated further. As frequent horizontal transfer of SCCmec and other mobile genetic elements seems to be the source of the ongoing genetic diversification of MRSA (Hallin et al., 2007; Lindsay et al., 2006; Robinson & Enright, 2003), comparative prevalence studies of large MSSA and MRSA populations should be conducted in parallel with MRSA surveillance studies to understand how natural populations of MSSA and MRSA co-evolve and interact. Our results suggest that analysis of the relationship between toxin genotypes and the toxin gene-encoding profiles of mobile genetic elements has a possible role in determining superantigenic toxin genotypes in S. aureus.

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