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 ν Sa4 encoding *sec*, *sell* and *tst-1*, and type I ν Sa β 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*.

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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* (SCC*mec*) (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

Abbreviations: MRSA, meticillin-resistant *Staphylococcus aureus*; MSSA, meticillin-susceptible *Staphylococcus aureus*; SCC*mec*, staphylococcal cassette chromosome *mec*; SE, staphylococcal enterotoxin.

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 V β 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, SEJ, SEK, SEL, 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 methicillin-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 Hiroasaki 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 1. Type of specimen and category of hospitalization of 118 patients with MRSA and 140 patients with MSSA infection or colonization

Source of clinical strain	No. of patients with MRSA (%) (n=118)	No. of patients with MSSA (%) (n=140)	Total (n=258)
Hospital ward/department			
Surgery	46 (38.1)	28 (20.0)	74
Internal medicine	18 (15.3)	20 (14.3)	38
Paediatrics	16 (13.6)	45 (32.1)	61
Urology	7 (5.9)	2 (1.4)	9
Dermatology	13 (11.0)	8 (5.7)	21
Obstetrics and gynaecology	2 (1.7)	8 (5.7)	10
Otolaryngology	13 (11.0)	20 (14.3)	33
Other	3 (2.5)	9 (6.4)	12
Source/body site			
Cannula and drain	7 (5.9)	11 (7.9)	18
Pharynx viscous liquid	14 (11.9)	25 (17.9)	39
Expectoration	33 (28.0)	23 (16.4)	56
Nasal cavity	2 (1.7)	5 (3.6)	7
Absorption slime in lung	3 (2.5)	15 (10.7)	18
Discharge from ear	9 (7.6)	7 (5.0)	16
Blood	13 (11.0)	7 (5.0)	20
Wound	6 (5.1)	9 (6.4)	15
Cellulitis and abscess	22 (18.6)	20 (14.3)	42
Faeces and urine	9 (7.6)	18 (12.9)	27

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 A_{260} 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, primers specific to the *S. aureus* *femA* and *femB* genes were used (Mehrotra *et al.*, 2000; Perez-Roth *et al.*, 2001). To construct a multiplex PCR system, four sets (set 1 – *sea*, *seb*, *sec*, *sed*, *see*, *femB*; set 2 – *seh*, *sei*, *selj*, *seip*, *femA*; set 3 – *selk*, *selm*, *selo*, *tst-1*, *femA*; set 4 – *sell*, *seln*, *selr*, *femB*) of 10 × primer master mixes containing 2 mM each primer were prepared.

Multiplex PCR for detection of *se* and *tst* genes. Multiplex PCR was performed using a multiplex PCR kit (Qiagen) according to the manufacturer's instructions. Each reaction mix (50 µl) consisted of 25 µl 2 × Qiagen multiplex PCR master mix (containing HotStart *Taq* DNA polymerase, multiplex PCR buffer and dNTP mix), 0.2 µM each primer and 10–100 ng template DNA. DNA amplification was carried out with the following thermal cycling regime: initial 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 superantigen genes tested. All of the MRSA isolates harboured more than one superantigen gene except for three strains (2.5%), which contained *seip* only (Table 2). Of the MRSA isolates, 44.1% and 17.8% contained eight and nine superantigen genes, respectively (Table 2, Fig. 1). Twelve strains (10.2%) contained 10 or 11 superantigen 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 superantigen 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 superantigen genes, although seven (5.0%) strains contained eight genes (Fig. 1). These results indicated that MRSA isolates harboured more superantigen genes than MSSA isolates.

We then compared the genotypes and frequency of superantigenic toxin genes in the MRSA and MSSA isolates. All superantigen 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

<i>S. aureus se</i> genotype	Prevalence			Suspected genomic island and plasmid
	MRSA (<i>n</i> =118)	MSSA (<i>n</i> =140)	Total (<i>n</i> =258)	
<i>sea</i>	—	7	7	ϕ Sa3mu
<i>sea, seg</i>	—	1	1	ϕ Sa3mu + <i>seg</i>
<i>sea, sec, sell</i>	—	1	1	ϕ Sa3mu, type II vSa3
<i>sea, sec, selo</i>	1	—	1	ϕ Sa3mu + <i>sec</i> + <i>seo</i>
<i>sea, seb, selk, selq</i>	—	1	1	ϕ Sa3mu, vSa1 (SaPI3)
<i>sea, sec, selo, selp</i>	2	—	2	ϕ Sa3mu, ϕ Sa3n + <i>sec</i> + <i>seo</i>
<i>sea, seg, sei, tst-1</i>	—	3	3	ϕ Sa3mu + <i>seg</i> + <i>sei</i> + <i>tst-1</i>
<i>sea, seh, selk, selq</i>	—	11	11	ϕ Sa3mw + <i>seh</i>
<i>sea, seg, sei, seln, tst-1</i>	—	7	7	ϕ Sa3mu + <i>seg</i> + <i>sei</i> + <i>sen</i> + <i>tst-1</i>
<i>sea, seg, sei, selm, seln, selo</i>	1	—	1	ϕ Sa3mu, type I vSa β
<i>sea, sec, seg, sell, seln, selo, tst-1</i>	1	—	1	ϕ Sa3mu, type I vSa4 + <i>seg</i> + <i>sen</i> + <i>seo</i>
<i>sea, sec, seg, sei, sell, selm, seln, selo, tst-1</i>	8	—	8	ϕ Sa3mu, type I vSa4, type I vSa β
<i>sea, sec, seg, sei, sell, selm, seln, selo, selp, tst-1</i>	10	—	10	ϕ Sa3mu, type I vSa4, type I vSa β , ϕ Sa3n
<i>seb</i>	—	1	1	
<i>seb, selp</i>	—	4	4	ϕ Sa3n + <i>seb</i>
<i>seb, selk, selq</i>	—	2	2	vSa1 (SaPI3)
<i>seb, seg, sei, selm, seln</i>	—	1	1	
<i>seb, seg, sei, selk, selm, seln, selo, selp, selq</i>	3	—	3	vSa1 (SaPI3), type I vSa β , ϕ Sa3n
<i>seb, sec, seg, sei, sell, selm, seln, selo, selp, tst-1</i>	1	—	1	Type I vSa4, ϕ Sa3n, type I vSa β + <i>seb</i>
<i>sec, sell, selp</i>	—	3	3	Type II vSa3, ϕ Sa3n
<i>sec, seg, sei, selo</i>	1	—	1	
<i>sec, sell, selp, tst-1</i>	1	—	1	Type I vSa4, ϕ Sa3n
<i>sec, seg, sei, sell, seln, selo</i>	—	2	2	Type II vSa3 + <i>seg</i> + <i>sei</i> + <i>sen</i> + <i>seo</i>
<i>sec, seg, sell, seln, selo, tst-1</i>	1	—	1	Type I vSa4 + <i>seg</i> + <i>sen</i> + <i>seo</i>
<i>sec, seg, sell, seln, selp, tst-1</i>	1	—	1	Type I vSa4, ϕ Sa3n + <i>seg</i> + <i>sen</i>
<i>sec, seg, sei, sell, selm, seln, selo</i>	—	8	8	Type II vSa3, type I vSa β
<i>sec, seg, sei, sell, selm, selo, tst-1</i>	4	—	4	Type I vSa4, type I vSa β
<i>sec, sei, sell, selm, selo, selp, tst-1</i>	1	—	1	Type I vSa4, ϕ Sa3n
<i>sec, seg, sei, sell, selm, seln, selo, tst-1</i>	52	3	55	Type I vSa4, type I vSa β
<i>sec, seg, sei, sell, selm, seln, selo, selp, tst-1</i>	10	—	10	Type I vSa4, type I vSa β , ϕ Sa3n
<i>sec, sed, seg, sei, selj, sell, selm, seln, selo, selr, tst-1</i>	1	—	1	Type I vSa4, pIB485, type I vSa β
<i>sed, seg, sei, selj, selm, seln, selo, selr</i>	—	4	4	pIB485, type I vSa β
<i>sed, selj, selr</i>	2	—	2	pIB485
<i>seg, sei</i>	—	1	1	
<i>seg, sei, selm</i>	—	1	1	
<i>seg, sei, seln</i>	—	1	1	
<i>seg, sei, tst-1</i>	—	1	1	
<i>seg, sei, selm, seln</i>	—	10	10	
<i>seg, sei, selm, seln, selo</i>	7	18	25	Type I vSa β
<i>seg, sei, selm, seln, selo, selp</i>	1	2	3	Type I vSa β , ϕ Sa3n
<i>selj, selr</i>	1	—	1	pF5
<i>selm, selo</i>	5	1	6	
<i>selo</i>	—	2	2	
<i>selp</i>	3	10	13	ϕ Sa3n
Negative for <i>se</i> genes	—	34	34	

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

Fig. 1. Numbers of superantigenic toxin genes found in MRSA or MSSA isolates collected from clinical specimens. Total DNA from *S. aureus* was purified, and four primer sets were used to anneal to unique regions and generate amplicons that allowed identification of each *se* or *tst* gene based on the molecular mass of its PCR product. The number of positive genes in each isolate was recorded. Black bars, MRSA; grey bars, MSSA.

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. *etb*, *sed*), phages

Fig. 2. Distribution of different superantigenic toxin genes among clinical MRSA and MSSA isolates. Total DNA from *S. aureus* was purified, and four primer sets were used to anneal to unique regions and generate amplicons that allowed identification of each *se* or *tst* gene based on the molecular mass of its PCR product. Overall numbers of each toxin gene in the MRSA or MSSA isolates were recorded and the percentage was calculated from the total number of isolates. Black bars, MRSA; grey bars, MSSA; neg, negative for toxin genes.

Fig. 3. Percentages of the various mobile genetic elements in MRSA and MSSA isolates. Mobile genetic elements encoding superantigenic toxin genes were analysed (see Table 2). Black bars, MRSA; grey bars, MSSA.

(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 allotyping 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 transposase 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 *seb* 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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