Exotoxin gene backgrounds in bloodstream and wound Staphylococcus aureus isolates from geriatric patients attending a long-term care Spanish hospital

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The exotoxin gene content was established for 62 Staphylococcus aureus isolates causing bloodstream (n=31) and wound (n=31) infections in geriatric patients attending a long-term care Spanish hospital from 1996 to 2006. Content was determined based on PCR screening of genes encoding five haemolysins, three exfoliatins, three leukotoxins and 21 pyrogenic toxin superantigens (PTSAgs), in addition to markers of genomic (SaPIs) and pathogenicity (SaPIs) islands. Exotoxin genes were abundant in both bloodstream (11–23 genes) and wound (8–19 genes) isolates, and they were arranged in 55 combinations with only two represented in both groups. All isolates were positive for genes encoding haemolysins (hl; 3–5) and PTSAgs (tst, se and sel; 5–14), whereas exfoliatin (et) and leukotoxin (luk) genes appeared in 98.4 and 51.6 % of isolates, respectively. The hlg, lukPV, tst, sec and selu genes were found significantly more frequently in bloodstream than in wound isolates, whereas hlg-variant, sea, seb, see and selk-selq were more frequent in wound isolates (P<0.05). Distinctive exotoxin gene combinations could potentially be associated with specific mobile genetic elements, including genomic islands [lukED, egc1 (seg, seln, sei, selm, selo) and egc2 (seg, seln, selu, sei, selm, selo)]; pathogenicity islands (etd, seb, sec, sell, selq, selk and tst); bacteriophages (eta, lukPV, sea, selp, selk, selq and see); and plasmids (sed, selj, ser, ses and sel). The abundance of exotoxin genes and variety of arrangements shown by Staphylococcus aureus from geriatric patients could play a role in the adaptation of the pathogen.

INTRODUCTION

Staphylococcus aureus has long been recognized as an important human pathogen causing disease in both hospitals and the community. The relative importance of host factors versus bacterial virulence determinants in the outcome of the disease is not well known, but it is accepted that bacterial components and products, including the capsule, surface-associated adhesins, secreted proteins and exotoxins, play a role in the process (Ferry et al., 2005). The vast majority of S. aureus isolates produce cytotoxins, such as haemolysins (encoded by hl genes), as well as enzymes, whose main function is to convert local host tissues into nutrients required for bacterial growth and to promote the spread of the pathogen through the human body. Some isolates also secrete other toxins, like bicomponent leukotoxins (LukS–LukF, encoded by luk genes) (Kaneko & Kamio, 2004), exfoliatins (ET, et genes) (Nishifuji et al., 2008), toxic shock syndrome toxin (TSST-1, tst gene), enterotoxins (SEs, se genes) and enterotoxin-like toxins (SEs, sel genes), with TSST-1, SEs and SEs belonging to the pyrogenic toxin superantigens (PTSAgs) (Larkin et al., 2009; Argudín et al., 2010). Exotoxin-encoding genes are variably represented in S. aureus isolates and most are carried by mobile genetic elements (MGEs), including genomic islands (SaPIs), pathogenicity islands (SaPIs), prophages and plasmids (Novick & Subedi, 2007; Baba et al., 2008; Ono et al., 2008; Goerke et al., 2009).

Specific exotoxins appear to be responsible for some syndromes caused by S. aureus. For instance, scalded skin syndrome has been associated with ETA and ETB (Nishifuji et al., 2008), toxic shock syndrome with TSST-1, SEB and SEC (Dinges et al., 2000), food poisoning with SEA (Dinges et al., 2000), haemolytic pneumonia and skin and soft tissue infections with LukPV (Lina et al., 1999) and allergic

Abbreviations: CC, clonal complex; DI, discrimination index; MGE, mobile genetic element; MLST, multilocus sequencing typing; MNH, Monte Naranco Hospital; MRSA, meticillin-resistant S. aureus; MSSA, meticillin-sensitive S. aureus; PTSAg, pyrogenic toxin superantigen.
syndromes, including asthma, chronic rhinitis and dermatitis, with SEB (Bachert et al., 2008). However, other S. aureus diseases are probably due to the cumulative effects of multiple virulence factors, and the precise contribution of different exotoxins to clinical presentation remains largely unknown (Peacock et al., 2002; Becker et al., 2003). To address this question, a better knowledge of the exotoxin gene content of S. aureus from different sources and associated with different types of infection is required. In the present work, the exotoxin gene profiles of S. aureus cultured from bloodstream and infected wounds of geriatric patients attending a long-term care Spanish hospital from 1996 to 2006 (Argudín et al., 2011a) were compared. The association of individual genes and gene combinations with known MGEs was inferred and their distribution between meticillin-resistant S. aureus (MRSA) and meticillin-sensitive S. aureus (MSSA), and between clonal complexes (CCs), was established.

**METHODS**

**Bacterial strains.** Sixty-two S. aureus isolates, collected in the Monte Naranco Hospital (MNH) over the 1996–2006 period (Argudín et al., 2011a), were analysed for virulence gene content and for the correlation of certain genes or gene combinations with known MGEs. All isolates involved in bloodstream infections (n=31, each from a different patient; B-group) and an equal number of isolates collected from patients with infected wounds, associated with chronic vascular conditions or traumatological surgery (W-group), were included in the study. The same isolates have been characterized previously by staphylococcal protein A gene (spa) typing, multilocus sequencing typing (MLST), accessory gene regulator system and meticillin resistance (Argudín et al., 2011a). The frequency of MRSA was 16.1 % (5 of 31) and 51.6 % (16 of 31) for bloodstream and wound isolates, respectively.

**PCR amplification.** Whole DNA was used as a template was extracted by the conventional protocol reported by Sambrook & Russell (2000) with lysostaphin (20 μg ml⁻¹; Sigma-Aldrich). Detection of exotoxin genes was achieved by PCR using primers and protocols reported previously for genes encoding haemolysins (hla, hlb, hld, hlg, hlgv), exfoliatins (eta, etb, etd), leukotoxins (lukED, lukM, lukPV), toxic shock syndrome toxin (Sts), SEs (sea, seb, sec, sed, see, seg, seh, sei, ser) and SELs (sel, selk, selm, seln, seb, selp, selp, selu), and for markers of SaPIs (ear), vSaB types I, II and III (splF), and vSaA type II (bsaB) (Argudín et al., 2009, 2011b). Oligonucleotides for the novel sel and set enterotoxin-encoding genes were designed for this study: ses-1 (5′-AATGCAATTGGCCCGATAG-3′); ses-2 (5′-AATCTCATATCCGAGGACA-3′); set-1 (5′-CGGGTGTACCTCTGTGATG-3′); and set-2 (5′-ATGTAATGCTTGGGACAT-3′). Amplification was performed using Tag DNA polymerase (Roche Diagnostics) and a Perkin-Elmer Gene Amplification PCR system (model 9600) programmed as follows: initial step of 95 °C for 5 min; 30 cycles of 95 °C for 30 s, 55 °C for 30 s and 72 °C for 30 s; followed by a final extension step of 72 °C for 5 min. The expected amplicons were of 214 and 162 bp for ses and set, respectively. Positive and negative controls were included in all PCR assays (Fuego et al., 2005a; Argudín et al., 2009, 2011b).

**Statistical methods.** For each trait, statistical comparison between B- and W-isolates was performed using the χ² test. Differences between groups were considered statistically significant if P-values were <0.05. The discrimination index (DI; the probability that two unrelated isolates would be assigned to different virulence profiles) was calculated using Simpson’s index of diversity (Struelsen, 1996).

**RESULTS**

The S. aureus isolates were screened for genes encoding 32 exotoxins, including five haemolysins, three exfoliatins, three leukotoxins and 21 PTSAgs (Table 1). All isolates were positive for three to five haemolysins, and all except one carried the leukotoxin lukED gene, 27.4 % carried lukPV, and lukM was the only gene absent in all isolates. With regard to exfoliatin genes, eta and etd appeared at similar frequencies (25.8 and 29.0 %, respectively), whereas etb was less common (4.8 %). Between five and 14 PTSAgs genes were detected in each isolate. All carried an enterotoxin gene cluster (egc1 or egc2, including seg, sei, selm, seln, selo + selu), all except three were positive for a classic enterotoxin gene (sea, seb, sec, sed) and 29.0 % carried tst.

As shown in Table 1, hlg, lukPV, tst, sec and selu were significantly more frequent in B- than in W-isolates, whereas hlgv, sea, seb, see, selk and selq were significantly more frequent in W- than in B-isolates (P<0.05). When total MRSA and MSSA isolates were compared (n=21 and 41, respectively), significant differences were found for etd, selk and selq, which were more frequent in MRSA, and for hlg, eta and tst, which were more frequent in MSSA. In all, exotoxin genes were abundant in both B- (11–23 genes) and W- (8–19 genes) isolates, and were arranged in 55 combinations (Tables 2 and 3). Thus, typing based on exotoxin gene content was highly discriminative, yielding a DI of 0.996.

To investigate possible associations between exotoxin gene(s) and MGEs, the bsaB, splF and ear genes were also screened as markers of vSaB types I, II and III, and SaPIs. The bsaB, splF and ear genes appeared in 16.0, 58.0 and 35.5 % of B-isolates and in 58.0, 87.1 and 96.8 % of W-isolates, respectively (Table 1). Distinct combinations of splF, bsaB, lukED and egc clusters (Table 4), detected at variable frequencies in the B-group compared with the W-group, could be correlated with vSaB type I (splF, lukED and egc1; 25.8 vs 35.5 %), and type III (splF and egc2; 25.8 vs 0 %). However, all isolates with the latter genes carried the lukED gene, which has not been reported in vSaB type III, and the splF, bsaB, lukED combination associated with vSaB type II (6.5 vs 48.4 %) co-existed with the egc1 or egc2 found in vSaB types I and III, respectively. These new combinations could correspond to unreported MGEs or to variants of those already known. Other gene arrangements were compatible with SaPI2 (tst; 58.0 vs 0 %), SaPI3 (ear, seb, selq and selk; 0 vs 9.7 %), SaPI5 (ear, selq and selk; 0 vs 9.7 %), SaPlm1/SaPlm1 (sec and tst; 9.7 vs 0 %), SaPImw2 (ear, sel and sec; 16.1 vs 3.2 %) and etdSaPI (32.3 vs 25.8 %).

The eta, lukPV, sea, selk, selq and selp genes are known to be carried by a variety of prophages of the family Siphoviridae,
Table 1. Distribution of exotoxin genes, and SaPIs and vSaβ markers, among MRSA and MSSA isolates recovered from blood and wound infections affecting geriatric patients (1996–2006)

Statistically significant results are highlighted in bold. NA, P-value not applicable; −, negative for the indicated gene or marker.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Total no. of isolates (%)</th>
<th>Total no. of B-isolates (%)</th>
<th>Total no. of W-isolates (%)</th>
<th>P-value</th>
<th>No. of MRSA isolates (%)</th>
<th>No. of MSSA isolates (%)</th>
<th>P-value</th>
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<td>16 (76.2)</td>
<td>24 (58.5)</td>
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<td>41 (100)</td>
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<tr>
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<td>0.31</td>
<td>−</td>
<td>1 (2.5)</td>
<td>0.47</td>
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<td>30 (96.8)</td>
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<td>16 (76.2)</td>
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<td>&lt;0.01</td>
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<td>12 (29.3)</td>
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</tbody>
</table>

DISCUSSION

In this study, the exotoxin gene content was established for a collection of well-characterized S. aureus isolates obtained from geriatric patients with bloodstream or wound infections (Argudín et al., 2011a). A high number of exotoxin genes was carried by each isolate, independently of whether they were recovered from blood (11–23 genes) or wounds (8–19 genes). Numerous virulence genes were also reported in S. aureus from other hospitals (Monk et al., 2004; Campbell et al., 2008; Holtfreter et al., 2007; Argudín et al., 2009; Varshney et al., 2009). In the present work, the detected genes appeared in many different combinations, with almost every isolate showing a distinct pattern. In agreement with this, typing based on exotoxin gene content assigned to subgroups Sa1, Sa2 and Sa3 based on integrase gene polymorphism (Table 4). These prophages were present in different percentages of B- and W-isolates: ΦSa1 (eta, 32.3 vs 16.1%), ΦSa2 (lukPV, 38.7 vs 16.1%), ΦSa3 (sea, 41.9 vs 83.9%); selp, 9.7 vs 9.7%; selk, selq and sea, 3.2 vs 19.4%). The see gene (0 vs 12.9%) could also be carried by a prophage. Six bloodstream and five wound isolates were positive for sed-selj, combined either with ser (10 isolates) or ses and set (one isolate). These enterotoxin gene combinations have been reported in pLB485-like and pF5-like plasmids, respectively (Table 4). Finally, set without ear-selq-selk (0 vs 9.7%), etb (9.0% vs 0%) and seh (6.5 vs 0%) could be located on plasmids pZA10 and pETB, and on the MGEw2/mssa476 element, respectively (Table 4).
several exotoxin genes, including between invasive and non-invasive isolates were found for 1608 Journal of Medical Microbiology was highly discriminative, even more so than spa typing (0.99 vs 0.98; Argudínez et al., 2011a). Despite the potential instability of genes carried by MGEs, exotoxin gene profiling could still be useful for short-term epidemiology in particular settings.

All isolates tested in this study were found to contain either egc1 or egc2, with the latter being almost completely restricted to the B-group. In fact, only one W-isolate, also recovered from blood of the same patient (Argudínez et al., 2011a), carried egc2. In addition, all isolates except one were positive for lukED, hla and hld. High frequencies of lukED and egc were also observed in S. aureus from a general university hospital located in the same city (Argudínez et al., 2009). Interestingly, significant differences between invasive and non-invasive isolates were found for several exotoxin genes, including hlg, lukPV, tst, sec and selu (more frequent in B-isolates), and for hlg-v, sea, seb, see and selk-selg (more common in W-isolates). As far as we know, this is the first comparison of the prevalence of exotoxin genes in invasive and non-invasive S. aureus isolates in geriatric patients. However, a recent study has compared the enterotoxin gene content of S. aureus strains derived from blood and wound infections affecting a heterogeneous population in New York (Varshney et al., 2009). In the study of Varshney et al. (2009), a high number of se genes arranged in many different combinations was also observed, but the prevalence rates in blood and wound isolates were comparable for most of the genes. In fact, only sed and selj in combination with ser were significantly more common in blood isolates. These genes, carried by pIB485-like plasmids, were infrequent in S. aureus from geriatric patients and distributed similarly between the two groups. In addition, selk was more common in MRSA and seh was more common in MSSA from New York, USA, whereas, in the present study, selk-selg and etd were significantly more frequent in MRSA, and hlg, eta and tst in the MSSA tested. The observed discrepancies may be due to differences in age, ethnicity and/or geographical origin of the patients: Spanish geriatric

Table 2. Exotoxin gene profiles of S. aureus isolates recovered from blood

- Negative for the markers tested.

<table>
<thead>
<tr>
<th>Isolate/year (CC)*</th>
<th>Virulence profile</th>
<th>Exotoxin genes</th>
<th>vSa///SaPI markers</th>
</tr>
</thead>
<tbody>
<tr>
<td>C16/96 (CC5)</td>
<td>V1</td>
<td>hla/b/d/gv-lukED/PV-eto-sea/c/lp-egc2</td>
<td>—</td>
</tr>
<tr>
<td>C17/97 (CC45)</td>
<td>V2</td>
<td>hla/g-lukED-sea/b/c/l-p-egc1</td>
<td>splF—ear</td>
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<tr>
<td>C18/97 (CC15)</td>
<td>V3</td>
<td>hla/d/gv-lukED-tst-sec-egc2</td>
<td>splF</td>
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<tr>
<td>C19/98 (CC45)</td>
<td>V4</td>
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<td>splF</td>
</tr>
<tr>
<td>C20/98 (CC8)</td>
<td>V5</td>
<td>hla/gv-lukED/PV-sea-egc1</td>
<td>splF—bsaB</td>
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<tr>
<td>C21/98 (CC45)</td>
<td>V6</td>
<td>hla/d/gv-lukED-tst-sec/l-egc1</td>
<td>ear</td>
</tr>
<tr>
<td>C22/98 (CC45)</td>
<td>V7</td>
<td>hla/d/gv-lukED-tst-sec/c/lp-egc2</td>
<td>ear</td>
</tr>
<tr>
<td>C24/99 (CC45)</td>
<td>V6</td>
<td>hla/d/gv-lukED-tst-sec/l-egc1</td>
<td>ear</td>
</tr>
<tr>
<td>C25/99 (CC15)</td>
<td>V8</td>
<td>hla/gv-gv-lukED-sec-egc1</td>
<td>splF</td>
</tr>
<tr>
<td>C26/99 (CC1)</td>
<td>V9</td>
<td>hla/d/gv-lukED/PV-eta/d-tst-sea/c/d/lj/fla/l/p-egc2</td>
<td>splF—bsaB</td>
</tr>
<tr>
<td>C27/00 (CC5)</td>
<td>V10</td>
<td>hla/d/gv-lukED/PV-eto-tst-sea/c/lj/r/t-egc2</td>
<td>bsaB</td>
</tr>
<tr>
<td>C28/00 (CC5)</td>
<td>V11</td>
<td>hla/d/gv-lukED-tst-sec/h-egc2</td>
<td>—</td>
</tr>
<tr>
<td>C29/00 (CC30)</td>
<td>V12</td>
<td>hla/d/gv-lukED-tst-sec-egc2</td>
<td>splF</td>
</tr>
<tr>
<td>C30/01 (CC121)</td>
<td>V13</td>
<td>hla/d/gv-lukED/PV-eta-tst-sec-egc2</td>
<td>splF</td>
</tr>
<tr>
<td>C31/01 (CC45)</td>
<td>V14</td>
<td>hla/d/gv-lukED-tst-sec-egc2</td>
<td>splF</td>
</tr>
<tr>
<td>C32/01 (CC45)</td>
<td>V15</td>
<td>hla/d/gv-lukED-tst-sec/c-egc2</td>
<td>splF</td>
</tr>
<tr>
<td>C33/01 (CC5)</td>
<td>V16</td>
<td>hla/d/gv-lukED-tst-sec-c-egc2</td>
<td>splF</td>
</tr>
<tr>
<td>C34/03 (CC45)</td>
<td>V17</td>
<td>hla/d/gv-lukED-sec-egc2</td>
<td>—</td>
</tr>
<tr>
<td>C35/01 (CC5)</td>
<td>V18</td>
<td>hla/d/gv-lukED-tst-sec-c-egc1</td>
<td>splF</td>
</tr>
<tr>
<td>C36/02 (CC5)</td>
<td>V19</td>
<td>hla/d/gv-lukED-sec/d/lj/r-egc2</td>
<td>splF—ear</td>
</tr>
<tr>
<td>C38/02 (CC88)</td>
<td>V20</td>
<td>hla/d/gv-lukED-tst-sec-egc2</td>
<td>splF</td>
</tr>
<tr>
<td>C39/02 (CC88)</td>
<td>V21</td>
<td>hla/d/gv-lukED/PV-eta/b-tst-sec/c-egc1</td>
<td>ear</td>
</tr>
<tr>
<td>C40/02 (CC88)</td>
<td>V22</td>
<td>hla/d/gv-lukED/PV-eta/b-tst-sec/c-egc1</td>
<td>splF—ear</td>
</tr>
<tr>
<td>C42/03 (CC45)</td>
<td>V23</td>
<td>hla/d/gv-lukED/PV-ll-egc2</td>
<td>splF—ear</td>
</tr>
<tr>
<td>C43/03 (CC45)</td>
<td>V24</td>
<td>hla/d/gv-lukED/PV-ll-egc1</td>
<td>ear</td>
</tr>
<tr>
<td>C44/03 (CC8)</td>
<td>V25</td>
<td>hla/d/gv-lukED/PV-eto-sea/c/d/lj/r-egc2</td>
<td>bsaB</td>
</tr>
<tr>
<td>C47/03 (CC5)</td>
<td>V26</td>
<td>hla/d/gv-lukED/PV-eto-tst-sec-egc2</td>
<td>splF</td>
</tr>
<tr>
<td>C50/04 (CC5)</td>
<td>V27</td>
<td>hla/d/gv-lukED-tst-sec/c/d/lj/fp-egc2</td>
<td>ear</td>
</tr>
<tr>
<td>C51/04 (CC5)</td>
<td>V28</td>
<td>hla/d/gv-lukED/PV-eto-seb/c/d/lj/r-egc1</td>
<td>splF</td>
</tr>
<tr>
<td>C53/05 (CC5)</td>
<td>V29</td>
<td>hla/d/gv-lukED/PV-eto-tst-sec/c/lp-egc1</td>
<td>ear</td>
</tr>
<tr>
<td>C57/06 (CC25)</td>
<td>V30</td>
<td>hla/d/gv-lukED-tst-egc2</td>
<td>bsaB</td>
</tr>
</tbody>
</table>

*MRSA isolates are shown in bold. CCs (Argudínez et al., 2011a) are shown in parentheses for each isolate.

Table 2 continues...
patients in the MNH vs heterogeneous (hispanic, black, caucasian and unknown) patients of different ages from hospitals in New York. However, as indicated by Varshney et al. (2009), the abundance of exotoxin genes and diversity of arrangement demand the examination of very large samples in order to establish confident correlations with clinical presentations. The present study included all invasive S. aureus isolates recovered from geriatric patients in the MNH over one decade, and an equal number of W-isolates. As the number of isolates was relatively low, the observed correlations must be taken with caution, but they can serve as a basis for further evaluation of virulence genes that might play a role in severe and less severe infections affecting geriatric patients.

MLST, supported by other typing techniques, has previously assigned the B- and W-isolates from the MNH to nine (CC1, CC5, CC8, CC15, CC25, CC30, CC45, CC88 and CC121), and seven (CC5, CC8, CC15, CC25, CC45, CC59 and CC72) CCs, respectively (Tables 2 and 3; Argudín et al., 2011a). Four of them (CC5, CC8, CC15 and CC45), out of a total of 11, included 80.6 % of the isolates, and were represented in both groups. The distribution of exotoxin genes and exotoxin gene combinations associated with known MGEs between CCs is shown in Table 4. Although most of the detected associations have been reported previously (Baba et al., 2002; Monk et al., 2004; Gill et al., 2005; Holtfreter et al., 2007; Tristan et al., 2004; Argudín et al., 2009; Ghebremedhin et al., 2009; Goerke et al., 2009; Peck et al., 2009; Varshney et al., 2009), new correlations were found in the present work. These include the association of CC88 and CC121 with the tst-carrying SaPI2; CC45 with selq-selk, known to be located on SaPI3, SaPI5 and Sa3, -carrying prophage. It is of note that this is the first time –carrying SaPI2; CC45 with selq-selk, known to be located on SaPI3, SaPI5 and Sa3, -carrying prophage. It is of note that this is the first time

---

**Table 3.** Exotoxin gene profiles of *S. aureus* recovered from wounds

<table>
<thead>
<tr>
<th>Isolate/year (CC)*</th>
<th>Virulence profile</th>
<th>Exotoxin genes</th>
<th>SaPI/r/Saβ markers</th>
</tr>
</thead>
<tbody>
<tr>
<td>C258/96 (CC8)</td>
<td>V31</td>
<td>hla/d/gv-lukED-eta-sea/b-egc1</td>
<td>ear-splF-BSaB</td>
</tr>
<tr>
<td>C259/96 (CC45)</td>
<td>V32</td>
<td>hla/d/gv-lukED-sea/b/cll-egc1</td>
<td>ear-splF</td>
</tr>
<tr>
<td>C260/96 (CC5)</td>
<td>V33</td>
<td>hla/d/gv-lukED-sea/c-egc1</td>
<td>ear-splF</td>
</tr>
<tr>
<td>C261/96 (CC8)</td>
<td>V34</td>
<td>hla/d/gv-lukED-sea/c-egc1</td>
<td>ear-splF-BSaB</td>
</tr>
<tr>
<td>C262/96 (CC8)</td>
<td>V35</td>
<td>hla/d/gv-lukED-sea/lk/lq-egc1</td>
<td>ear-splF-BSaB</td>
</tr>
<tr>
<td>C263/96 (CC8)</td>
<td>V36</td>
<td>hla/d/gv-lukED-sea/lk/lq-egc1</td>
<td>ear-splF-BSaB</td>
</tr>
<tr>
<td>C264/96 (CC59)</td>
<td>V37</td>
<td>hla/d/gv-lukED-sea/lk/lq-egc1</td>
<td>ear-splF-BSaB</td>
</tr>
<tr>
<td>C265/96 (CC5)</td>
<td>V38</td>
<td>hla/d/gv-lukED-sea/b-egc1</td>
<td>ear-splF-BSaB</td>
</tr>
<tr>
<td>C266/97 (CC8)</td>
<td>V39</td>
<td>hla/d/gv-lukED-sea/c-egc1</td>
<td>ear-splF-BSaB</td>
</tr>
<tr>
<td>C267/97 (CC5)</td>
<td>V40</td>
<td>hla/d/gv-lukED-sea-bgc1</td>
<td>ear-splF</td>
</tr>
<tr>
<td>C268/97 (CC5)</td>
<td>V41</td>
<td>hla/d/gv-lukED-eta-sea/b/c-egc1</td>
<td>ear-splF-BSaB</td>
</tr>
<tr>
<td>C269/97 (CC5)</td>
<td>V42</td>
<td>hla/d/gv-lukED-sea/c-egc1</td>
<td>ear-splF-BSaB</td>
</tr>
<tr>
<td>C270/97 (CC8)</td>
<td>V43</td>
<td>hla/d/gv-lukED-sea/c-egc1</td>
<td>ear-splF</td>
</tr>
<tr>
<td>C271/98 (CC8)</td>
<td>V43</td>
<td>hla/d/gv-lukED-sea/c-egc1</td>
<td>ear-splF</td>
</tr>
<tr>
<td>C272/99 (CC15)</td>
<td>V8</td>
<td>hla/d/gv-lukED-sea/c-egc1</td>
<td>splF</td>
</tr>
<tr>
<td>C273/99 (CC45)</td>
<td>V44</td>
<td>hla/d/gv-lukED-sea/b/c/lk/lq-egc1</td>
<td>ear-splF-BSaB</td>
</tr>
<tr>
<td>C274/00 (CC8)</td>
<td>V37</td>
<td>hla/d/gv-lukED-sea/lk/lq-egc1</td>
<td>ear-splF-BSaB</td>
</tr>
<tr>
<td>C275/00 (CC5)</td>
<td>V45</td>
<td>hla/d/gv-lukED/PV-sea-egc1</td>
<td>ear-splF</td>
</tr>
<tr>
<td>C276/00 (CC5)</td>
<td>V36</td>
<td>hla/d/gv-lukED-sea/b/lk/lq-egc1</td>
<td>ear-splF</td>
</tr>
<tr>
<td>C277/01 (CC8)</td>
<td>V46</td>
<td>hla/d/gv-lukED/PV-sea-c-egc1</td>
<td>ear-splF</td>
</tr>
<tr>
<td>C278/01 (CC5)</td>
<td>V47</td>
<td>hla/d/gv-lukED/PV-sea/b/c/lk/lq-egc1</td>
<td>ear-splF-BSaB</td>
</tr>
<tr>
<td>C279/02 (CC5)</td>
<td>V48</td>
<td>hla/d/gv-lukED/PV-sea/b/c/lk/lq-egc1</td>
<td>ear-splF-BSaB</td>
</tr>
<tr>
<td>C280/02 (CC15)</td>
<td>V49</td>
<td>hla/d/gv-lukED/PV-sea/b/c/lk/lq-egc1</td>
<td>ear-splF-BSaB</td>
</tr>
<tr>
<td>C281/02 (CC15)</td>
<td>V50</td>
<td>hla/d/gv-lukED/PV-sea/b/c/lk/lq-egc1</td>
<td>ear-splF</td>
</tr>
<tr>
<td>C282/03 (CC8)</td>
<td>V51</td>
<td>hla/d/gv-lukED/PV-sea/b/c/lk/lq-egc1</td>
<td>ear-splF-BSaB</td>
</tr>
<tr>
<td>C283/03 (CC15)</td>
<td>V38</td>
<td>hla/d/gv-lukED/PV-sea/b/c/lk/lq-egc1</td>
<td>ear-splF-BSaB</td>
</tr>
<tr>
<td>C284/04 (CC15)</td>
<td>V52</td>
<td>hla/d/gv-lukED/PV-sea/b/c/lk/lq-egc1</td>
<td>ear-splF-BSaB</td>
</tr>
<tr>
<td>C285/06 (CC25)</td>
<td>V30</td>
<td>hla/d/gv-lukED/PV-sea/b/c/lk/lq-egc1</td>
<td>ear-splF-BSaB</td>
</tr>
<tr>
<td>C286/06 (CC5)</td>
<td>V53</td>
<td>hla/d/gv-lukED/PV-sea/b/c/lk/lq-egc1</td>
<td>ear-splF-BSaB</td>
</tr>
<tr>
<td>C287/06 (CC5)</td>
<td>V54</td>
<td>hla/d/gv-lukED/PV-sea/b/c/lk/lq-egc1</td>
<td>ear-splF-BSaB</td>
</tr>
<tr>
<td>C289/03 (CC72)</td>
<td>V55</td>
<td>hla/d/gv-egc1</td>
<td>splF</td>
</tr>
</tbody>
</table>

*MRSA isolates are shown in bold. CCs (Argudín et al., 2011a) are shown in parentheses for each isolate.
Foodstuffs and food-handlers have been tested (Fueyo et al., 2001, 2005a, b; Argudín et al., 2009). In other studies, the see gene was either not detected (Peacock et al., 2002; Hu et al., 2008) or uncommon (frequency of about 1%; Becker et al., 2003; Nashev et al., 2007; Baba-Moussa et al., 2008). However, 20–40% of S. aureus isolates associated with steroid-resistant atopic dermatitis, the vagina of a healthy woman and a general population of patients were recently reported to carry the see gene (Schlievert et al., 2008).

Some of the inferred MGEs, including vSaβ type I (lukED-egc1), vSaβ type II (lukED), vSaβ type III (egc2), or variants therein, SaPI2 (stas), etdSaPI, φSa1 (eta), φSa2 (lukPV) and

<table>
<thead>
<tr>
<th>Suspected MGE*</th>
<th>Total isolates (n/%)‡</th>
<th>CCs†</th>
<th>Bloodstream isolates (n)</th>
<th>Wound isolates (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>vSaβ I (pSPI-lukED-egc1)</td>
<td>CC5 (7/31.8), CC8 (2/16.7), CC15 (5/71.4), CC45 (4/44.4), CC88 (1/33.3)</td>
<td>CC5 (3), CC15, CC45 (3), CC88</td>
<td>CC5 (4), CC8 (2), CC15 (4), CC45</td>
<td></td>
</tr>
<tr>
<td>vSaβ II (pSPI-bsaB-lukED) + egc1</td>
<td>CC5 (5/22.7), CC8 (9/75.0), CC15 (1/14.3), CC45 (1/11.1)</td>
<td>CC8</td>
<td>CC5 (5), CC8 (8), CC15, CC45</td>
<td></td>
</tr>
<tr>
<td>vSaβ III (pSPI-egc2) + lukED</td>
<td>CC1 (1/100), CC5 (2/9.1), CC15 (1/14.3), CC30 (2/100), CC88 (1/33.3), CC121 (2/100)</td>
<td>CC5 (2), CC15, CC30 (2), CC88, CC121 (2)</td>
<td>CC5, CC8, CC45</td>
<td></td>
</tr>
<tr>
<td>SaPI2 (stas)</td>
<td>CC1 (1/100), CC5 (7/31.8), CC15 (1/14.3), CC30 (1/50), CC45 (3/33.3), CC88 (3/100), CC121 (2/100)</td>
<td>CC1, CC5, CC15, CC30, CC45 (3), CC88 (3), CC121 (2)</td>
<td>CC5, CC8, CC45</td>
<td></td>
</tr>
<tr>
<td>SaPI3 (ear-seb-selq-selk)§</td>
<td>CC5 (1/4.5), CC8 (1/8.3), CC45 (1/11.1)</td>
<td>CC1, CC5, CC15, CC30, CC45, CC88</td>
<td>CC5, CC8, CC45</td>
<td></td>
</tr>
<tr>
<td>SaPI5 (ear-ez-selk)§</td>
<td>CC8 (2/16.7), CC59 (1/100)</td>
<td>CC1, CC5, CC15, CC30, CC45, CC88</td>
<td>CC5, CC8, CC45</td>
<td></td>
</tr>
<tr>
<td>SaPIm1/SaPIm1 (sel-se-tst)‖</td>
<td>CC45 (3/33.3)</td>
<td>CC45 (3)</td>
<td>CC5, CC8, CC45</td>
<td></td>
</tr>
<tr>
<td>etdSaPI (etd)</td>
<td>CC45 (3/33.3)</td>
<td>CC45 (3)</td>
<td>CC5, CC8, CC45</td>
<td></td>
</tr>
<tr>
<td>φSa1 (eta)</td>
<td>CC1 (1/100), CC5 (6/27.3), CC8 (2/16.7), CC15 (1/14.3), CC45 (2/22.2), CC88 (1/33.3)</td>
<td>CC1, CC5 (3), CC8, CC45 (2), CC88 (2), CC121 (2)</td>
<td>CC5, CC8, CC45</td>
<td></td>
</tr>
<tr>
<td>φSa2 (lukPV)</td>
<td>CC1 (1/100), CC5 (7/31.8), CC8 (4/33.3), CC15 (1/14.3), CC45 (1/11.1), CC88 (2/66.7), CC121 (1/50)</td>
<td>CC1, CC5 (5), CC8 (2), CC45, CC88 (2), CC121 (2)</td>
<td>CC5, CC8, CC45</td>
<td></td>
</tr>
<tr>
<td>φSa3 (sea)</td>
<td>CC5 (14/63.6), CC8 (12/100), CC15 (4/57.1), CC30 (2/100), CC45 (4/44.4), CC88 (2/66.7), CC59 (1/100)</td>
<td>CC5 (5), CC8 (2), CC45 (2), CC88 (2)</td>
<td>CC5, CC8, CC45</td>
<td></td>
</tr>
<tr>
<td>φSa3 (sea-sek-selek)§</td>
<td>CC1 (1/100), CC5 (3/27.3)</td>
<td>CC1, CC5 (3)</td>
<td>CC5</td>
<td></td>
</tr>
<tr>
<td>φSa3 (sel)</td>
<td>CC5 (6/27.3)</td>
<td>CC5 (3)</td>
<td>CC5</td>
<td></td>
</tr>
<tr>
<td>φSa (see)</td>
<td>CC5 (3/13.6), CC15 (1/14.3)</td>
<td>CC1, CC5 (3), CC8</td>
<td>CC5 (3), CC15</td>
<td></td>
</tr>
<tr>
<td>plB458 family (sel-selk ± sel)</td>
<td>CC1 (1/100), CC5 (7/31.8), CC8 (2/16.7)</td>
<td>CC1, CC5 (3), CC8</td>
<td>CC5 (4), CC8</td>
<td></td>
</tr>
<tr>
<td>pF (sel-sel-sel-set)</td>
<td>CC5 (1/4.5)</td>
<td>CC5</td>
<td>CC5</td>
<td></td>
</tr>
<tr>
<td>pETB (etb)</td>
<td>CC5 (1/4.5), CC88 (2/66.7)</td>
<td>CC5, CC88 (2)</td>
<td>CC5</td>
<td></td>
</tr>
<tr>
<td>pZA10 (seb)</td>
<td>CC5 (5/22.7), CC88 (1/8.3), CC15 (4/57.1), CC45 (2/22.2)</td>
<td>CC5, CC45, CC88</td>
<td>CC5, CC8, CC45</td>
<td></td>
</tr>
<tr>
<td>MGEmw2/mssa476 (seb)</td>
<td>CC1 (1/100), CC5 (1/4.5)</td>
<td>CC1, CC5</td>
<td>CC1, CC5</td>
<td></td>
</tr>
</tbody>
</table>

*Genomic islands according to Baba et al. (2008); pathogenicity islands according to Novick & Subedi (2007) and Yamaguchi et al. (2002); prophage types according to Couch et al. (1988) and Goerke et al. (2009); plasmids according to Altboum et al. (1985), Bayles & Iandolo (1989), Fueyo et al. (2005b), Ono et al. (2008) and Yamaguchi et al. (2001); MGEmw2/mssa476 element according to Noto & Archer (2006).
†Clonal complexes according to Argudín et al. (2011a).
§Percentages calculated with respect to the total number of isolates within each CC: CC1 (1), CC5 (22), CC8 (12), CC15 (7), CC25 (2), CC30 (2), CC45 (9), CC59 (1), CC72 (1), CC88 (3), CC121 (2).
‖These isolates also carried sea and could be positive for φSa3 (sea) or φSa3 (sea-sek-selek).
¶These isolates were also positive for ear and could carry SaPIm2 (ear-ez-sekselek).
ΦSa3 (sea), were distributed widely, being present in five to seven CCs. Other SaPIs (such as SaP13, SaP15 and the sec-carrying SaPIs), prophages (sea-selk-self- and self-carrying ΦSa3, and the see-carrying ΦSa), the seh-carrying MGEs, as well as plasmids of the pIB458 and pF5 families, were restricted to one to three CCs. The basis for the different distribution of MGEs, and toxin genes therein, between *S. aureus* lineages remains unknown, but the high number and variety of exotoxin genes found in the nosocomial isolates characterized in this study, together with the expected differences in gene expression (Robinson et al., 2005; Varshney et al., 2009), could play a role in the adaptation of this highly versatile pathogen. Moreover, successful combinations of virulence genes may be among the factors leading to the increase in frequency of certain strains and their epidemic spread both within and outside the nosocomial setting.

**ACKNOWLEDGEMENTS**

This work was supported by the Spanish Ministry of Science and Innovation (project FIS-P10808656). M. A. A. was supported by the Ministry of Science and Innovation, Spain (grant FPU AP-2004-3641) co-funded by the European Social Fund.

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