Clonal Complexes and Diversity of Exotoxin Gene Profiles in Methicillin-Resistant and Methicillin-Susceptible

*Staphylococcus aureus* Isolates from Patients in a Spanish Hospital

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Molecular epidemiology studies have allowed the identification of the methicillin (meticillin)-resistant (MRSA) and methicillin-susceptible (MSSA) clonal complexes (CCs) and clones of *Staphylococcus aureus* circulating in a Spanish hospital recently. Of 81 isolates tested, 32.1% were MRSA. Most of them carried staphylococcal cassette chromosome mec (SCCmec) IVc (88.5%) and belonged to CC5 (88.5%); multilocus sequence typing types ST125 [mainly associated with spa type t067], ST5, and ST228. A higher diversity was found among MSSA isolates (67.9%). Eighty percent shared the genetic background of major MRSA lineages (CC5 [38.2%; ST125 and ST5], CC30 [25.5%; ST30], CC45 [14.5%; ST45 and ST47], and CC8 [1.8%; ST8]), but CC12, CC15, CC51, and CC59 were also detected. Many exotoxin genes were present in each of the 81 isolates, independent of whether they were involved in sepsis (11 to 22) or other types of infections (13 to 21), and they appeared in 73 combinations. The relevant data are that (i) all isolates were positive for hemolysin and leukotoxin genes (98.8% for lukED and 25.9% for lukP); (ii) all contained an enterotoxin gene cluster (egc with or without see), frequently with one or more genes encoding classical enterotoxins; (iii) about half were positive for tst and 95% were positive for exfoliatin-encoding genes (eta, etb, and/or etd); and (iv) the four agr groups were detected, with agrII (55.6%) and agrIII (23.5%) being the most frequent. Taken together, results of the present study suggest a frequent acquisition and/or loss of exotoxin genes, which may be mediated by efficient intralineage transfer of mobile genetic elements and exotoxin genes therein and by eventual breakage of interlineage barriers.

*Staphylococcus aureus* is both a commensal bacterium and an extremely versatile pathogen that causes a wide range of diseases in humans, including superficial, deep-seated, and systemic infections, as well as a variety of toxemic syndromes, such as toxic shock syndrome (TSS), staphylococcal scalded-skin syndrome (SSSS), and staphylococcal food poisoning (36). *S. aureus* produces a wide range of virulence factors that mediate host colonization, invasion of damaged skin and mucosa, dissemination through the body, and evasion of host defense mechanisms (8, 12). Relevant among them are a variety of exotoxins that comprise α-, β-, γ-, and δ-hemolysins, leukotoxins (the classical LukS-PV–LukF-PV Panton-Valentine leukocidin [LukPV], LukE-LukD [LukED] and LukM-LukF⁺-PV [LukM]), exfoliative toxins, and pyrogenic toxin superantigens, such as the staphylococcal TSS toxin (TSST-1, first referred to as SEF) and staphylococcal enterotoxins (SEs) (14, 29, 43). Five major serological types of SEs, SEA through SEE (known as classical enterotoxins, encoded by the see to see genes, respectively) have been initially identified. However, new types of SEs and their coding genes (seg through seeu) were later reported. Several SE genes (seg, sei, sem, sen, and seeu) are part of an operon termed the enterotoxin gene cluster (egc), of which a variant that contains see instead of the two pseudo-genes present in the originally described ege between sei and sen has been identified (27, 33). Both TSST-1 and SEs are potent activators of T-cell populations, leading to massive proliferation and uncontrolled release of proinflammatory cytokines (14). Expression of most virulence factors in *S. aureus* is under the control of the agr (accessory gene regulator) locus, which encodes a two-component signaling pathway and its activating ligand, a bacterial-density-sensing peptide termed the autoinducing peptide (37). *S. aureus* strains can be subdivided into four major agr groups, based on polymorphisms in the amino acid sequence of the autoinducing peptide and other components of the system (26, 28). Within a given group, each strain produces a peptide that can activate the agr response in other members of the group, whereas the autoinducing peptides produced by different groups are usually mutually inhibitory (26).

Apart from having pathogenic versatility, *S. aureus* can adapt rapidly to the selective pressure of antibiotics, with the emergence and spread of methicillin ( meticillin)-resistant *S. aureus* (MRSA) isolates being a relevant example. Resistance to methicillin and other beta-lactam antibiotics is caused by the mecA gene, situated on a mobile genetic element, the staphylococcal cassette chromosome mec (SCCmec), which consists of the mec gene complex, the ccr gene complex, and the “junkyard” regions. Based on the variability of the differently combined components, several types of SCCmec and several vari-
ants of the types have been distinguished (21, 23, 24, 25, 30, 31, 38, 45, 51).

In the present work, the techniques most commonly applied in epidemiological studies of *S. aureus* were used to identify the prevalent and sporadic MRSA and methicillin-susceptible *S. aureus* (MSSA) clones that have been causing disease in a Spanish hospital (the Hospital Universitario Central de Asturias [HUCA]) over a recent time period (2005 to 2006). These methods included pulsed-field gel electrophoresis (PFGE) of Smal-digested genomic DNA (Smal PFGE), *S. aureus* protein A gene (*spa*) typing, multilocus sequence typing (MLST) analysis, and SCCmec typing of MRSA (4, 9, 40, 42, 50, 52). The risk for human health posed by the accumulation of virulence genes in *S. aureus* (34) along with the potential application of such genes for subtyping prompted the assessment of the virulence gene repertoire of the HUCA isolates, with regard to the agr group and 30 exotoxin-encoding genes.

**MATERIALS AND METHODS**

*S. aureus* isolates. Eighty-one *S. aureus* isolates were analyzed in this study. Each was collected from a different patient attending the HUCA from December 2005 to December 2006. These isolates were identified in the hospital by use of standard procedures. Briefly, suspected colonies of *S. aureus* obtained in primary cultures were tested for agglutination with Pastorex Staph-plus (Bio-Rad Laboratories SA, Alcobendas, Madrid, Spain) and for thermonuclease (on DNase test agar; Biomedics, Madrid, Spain) and coagulase production (coagulase plasma; Becton Dickinson, San Agustín de Gualdalix, Madrid, Spain). Isolates that proved to be positive for the three tests were also evaluated for susceptibility or resistance to methicillin. *S. aureus* isolates were recovered from suppurative samples from spontaneous infections (5 isolates from patients with conjunctivitis, abscesses in the hand, umbilical pyogenic granuloma, parotiditis, or infected skin, surgical wounds (29 isolates associated with hand or leg amputations, hips, knee, or valvular protheses, varicose veins, cellulitis, artheroses, osteomyelitis, Morton’s neuroma, thoracotomy, tracheotomy, pleuritis [in two patients with esophageal cancer], or colon infection [in a patient with colon cancer]), urine (1 isolate from a patient with postcatheter urinary tract infection), tracheobronchial aspirates (11 isolates from patients with staphylococcal pneumonia, who have required intubation or assisted ventilation after cerebrovascular accident, brain injury, or polytraumatis), skin exudates (2 isolates from children with SSSS, 1 also involved in sepsis), and blood (33 isolates from patients who developed sepsis, most of them with severe underlying conditions, such as leukemia, stomach cancer, liver transplantation, cerebrovascular accidental, thyroidosis, aortic aneurysm, chronic renal insufficiency subjected to hemodialysis or peritoneal dialysis, pneumonia, and/or the presence of inserted catheters).

Macrorestriction-PFGE analysis. Whole DNA from each *S. aureus* isolate was analyzed by Smal PFGE using a CHEF-DRIII SYS220/240 (Bio-Rad Laboratories SA, Alcobendas, Madrid, Spain) system and the consensus protocol of the European S. aureus whole genome sequencing project (SAGUAS) [32, 33, 34] along with the potential application of the *agr* groups of *S. aureus* isolates. ST5, ST125, and ST228 belong to the same CC, namely, CC5, which clearly predominated in the HUCA.

With regard to the remaining Smal PFGE clusters, the following observations were made. (i) Representative isolates of cluster B showed *spa* types t012 (four out of five tested) and t021 (the remaining one), and one isolate of each type proved to be of ST30 within CC30. (ii) Isolates belonging to cluster D were of *spa* types t282 and t1618 (one and two out of three tested, respectively), and one of the latter isolates was of ST45. (iii) Isolates in cluster A were of *spa* types t081, t383, and t1494, and the t383 isolate was of ST47. Both ST45 and ST47 (which differs from ST45 by one change in the amplified region of *aroE* [9]) belong to CC45, although the corresponding PFGE profiles grouped separately in the dendrogram. (iv) Two *spa* types and one ST were associated with clusters E (t547 and t084; both ST15) and F (t160 and t1381; ST12). Finally, the S32, S12, and S19 unclustered Smal PFGE profiles were t024, t159, and nontypeable, respectively. The predicted ST for t024 is ST18 (CC8), t159 could be of ST121 or ST427 (CC51), and the last type was experimentally assigned to ST59 (CC59).

**RESULTS**

Genotypic typing of the isolates. For a detailed assessment of the epidemiology of *S. aureus* circulating in the HUCA, the 81 isolates selected for the present study were subjected to Smal PFGE, and representative subsets were also typed by *spa* typing and MLST.

Smal PFGE yielded a DI of 0.98 and identified 50 profiles (S1 to S50; Fig. 1), with only 15 including more than one isolate (two up to seven). On the basis of the coefficient of similarity of Jaccard, a dendrogram was constructed (Fig. 2). At a cutoff point of 0.64, two major clusters (B and G), five minor clusters (A, C, D, E, and F), and several ungrouped branches were revealed. Cluster G, which accounted for 43.2% of the isolates, could be in turn separated into subclusters G1 and G2. Thirteen isolates of cluster G were selected for *spa* typing (Table 1). Four of them, all belonging to G1, were assigned to t002, another two were assigned to t3734, and seven were assigned to t067, and these last nine were included in G2. MLST performed on one or two isolates of each *spa* type associated t002 with ST5, while the t067 and t3734 isolates were associated with ST125 (which differs from ST5 by only one base pair in the amplified region of the *yqiL* gene [46]). Interestingly, representative isolates of cluster C and the single S20 isolate that generated the unclustered branch closest to cluster G were also of *spa* type t067 and ST125, whereas the next branch included three S25 isolates that were of *spa* type t109 and ST228 (a two-locus variant of ST5, with 1- and 2-bp differences in the *tpi* and *yqiL* amplified regions, respectively [48]). ST5, ST125, and ST228 belong to the same CC, namely, CC5, which clearly predominated in the HUCA.

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Incidence of MRSA and SCCmec typing. Of the isolates under study, 55 were MSSA (67.9%) and 26 were MRSA (32.1%). The latter belonged to Smal PFGE clusters A, C, and
G or generated one of three unclustered patterns (S20, S25, and S48). However, more than half (16 out of 26) grouped in subcluster G2 (spa type t067 or t3734; ST125) within cluster G, and nearly all were members of CC5 (Fig. 2). All MRSA isolates contained SCCmec IVc, except two that carried SCCmec I and generated the unclustered S25 profile (spa type t109; ST228) and one that was nontypeable by the applied method (t067; subcluster G2).

**Virulence gene profiles.** The agr group and the exotoxin gene repertoire were determined for the 81 *S. aureus* isolates of the HUCA and for *S. aureus* NCTC 8325 (included as a control). A dendrogram of similarity constructed on the basis of the presence or absence of the screened genes separated the control strain from the HUCA isolates and revealed a wide heterogeneity among the latter (Fig. 3). In fact, they were distributed into 73 virulence gene profiles, with only six being shared by more than one isolate (two or three). Relevant data were as follows. (i) The four types of *agr* systems were represented in the series, with *agrII* being the most common (55.6%), followed by *agrIII* (23.5%), *agrIV* (12.3%), and *agrI* (8.6%). (ii) All isolates were beta-hemolytic and positive for *hla* and *hlg-2* (encoding α- and γ-variant hemolysins, respectively). A clearly prevalent profile (92.6%) included the five hemolysin genes tested (*hla*–*hlb*–*hld*–*hlg*–*hlg-2*). (iii) All isolates carried at least one leukotoxin-encoding gene, most frequently *lukED* (98.8%), but *lukPV* also occurred (25.9%). In contrast, *lukM* was not detected. (iv) A remarkable number of isolates (95.1%) was positive for genes encoding exfoliative toxins, *etb* (89.9%), found alone or in combination with *eta* and/or *etd*, was the most common. It was followed by *eta* and *etd*, carried by 43.2% and 17.3% of the isolates, respectively. Despite this, only two young children were diagnosed with SSSS, with one of
the responsible isolates being positive for etb, eta, and agrIV and the other being positive for etb and agrII. (v) About half of the isolates (51.9%) carried tst, although none of the patients suffered from TSS. (vi) All isolates contained an egc or an egc-like cluster (70.4% and 29.6%, respectively), frequently together with other SE genes, including those encoding classical enterotoxins (92.6%). Of the latter, sec, sea, seb, and sed were present in 80.2%, 55.6%, 27.2%, and 24.7% of the isolates, respectively. Each of these genes appeared either alone (in a few cases) or with one or two of the other in different combinations. All HUCA isolates were negative for see. The sed, sej, and ser genes carried by plasmids were present in 24.7% of the isolates, with the three coinciding in 17.3% of them. Among other SE genes, sep was the most frequent (24.7%), but seh, sek, sel, and seq were also represented (12.3%, 2.5%, 2.5%, and 3.7%, respectively). Interestingly, typing of the isolates on the basis of the virulence gene profile yielded a DI close to 1 (0.997).

Overall, a very high number of exotoxin genes was present in

TABLE 1. Relationships between CC, MLST type, spa type, and PFGE cluster

<table>
<thead>
<tr>
<th>CC</th>
<th>MLST type(s) (n)</th>
<th>spa type(s) (n)</th>
<th>PFGE cluster</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC5</td>
<td>ST5 (2)</td>
<td>t002 (4)</td>
<td>G1</td>
</tr>
<tr>
<td></td>
<td>ST125 (2)</td>
<td>t067 (7), t3734 (2)</td>
<td>G2</td>
</tr>
<tr>
<td></td>
<td>ST125</td>
<td>t067 (4)</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td>ST228 (ST125)</td>
<td>t109 (3)</td>
<td>–</td>
</tr>
<tr>
<td>CC8</td>
<td>(ST88)</td>
<td>t024</td>
<td>–</td>
</tr>
<tr>
<td>CC12</td>
<td>ST12</td>
<td>t160, t1381</td>
<td>F</td>
</tr>
<tr>
<td>CC15</td>
<td>ST15 (2)</td>
<td>t084 (2), t547</td>
<td>E</td>
</tr>
<tr>
<td>CC30</td>
<td>ST30 (2)</td>
<td>t012 (4), t021</td>
<td>B</td>
</tr>
<tr>
<td>CC45</td>
<td>ST45</td>
<td>t282, t1618 (2)</td>
<td>D</td>
</tr>
<tr>
<td></td>
<td>ST47</td>
<td>t081 (2), t183, t1494</td>
<td>A</td>
</tr>
<tr>
<td>CC51</td>
<td>(ST121, ST427)</td>
<td>t159</td>
<td>–</td>
</tr>
<tr>
<td>CC59</td>
<td>ST59</td>
<td>NT</td>
<td>–</td>
</tr>
</tbody>
</table>

* The numbers of isolates (n) are listed in parentheses if the number is more than one.

* Determined or presumptive (according to the spa type; in parentheses) STs of representative isolates are shown.

* NT, nontypeable.

* –, unclustered PFGE profile.

FIG. 2. Dendrogram showing the relatedness between SmaI macrorestriction fragment profiles generated from the S. aureus clinical isolates and the control strain (NCTC 8325). At a Jaccard coefficient of similarity (J) of 0.64, seven clusters (labeled A to G, the latter with subclusters G1 and G2) and several unclustered branches were detected. Footnote a, determined multilocus sequence types of representative isolates; footnote b, MSSA and MRSA. n, number of isolates.
FIG. 3. Dendrogram showing the relatedness between virulence gene profiles (for 30 exotoxin genes and the agr group) of the S. aureus isolates tested. At a Jaccard coefficient of similarity (J) of ca. 0.63, two main clusters, termed V1 and V2, and three subclusters within V2 (V2a, V2b, and V2c) are indicated. The type of infection caused by each isolate, the Smal PFGE cluster to which it belongs, the SCCmec type (I, IVc, or not typeable [nt]) of MRSA, and the spa type as well as the MLST type of representative isolates, are shown at the right of the dendrogram.
TABLE 2. Characteristics of the virulence clusters and subclusters.a

<table>
<thead>
<tr>
<th>Virulence cluster (n)</th>
<th>agr type (n)</th>
<th>CC (n)</th>
<th>Cytotxin(s) (n)b</th>
<th>Pyrogenic toxin superantigen gene(s) (n)b</th>
</tr>
</thead>
<tbody>
<tr>
<td>V1</td>
<td>agrI (4)</td>
<td>CC8, CC30, CC45</td>
<td>lukED (4), lukPV eta (3), eta (4), etd (2)</td>
<td>tst (2), sea (2), sed (4), sej (3), ser (3)</td>
</tr>
<tr>
<td></td>
<td>agrII (12)</td>
<td>CC5 (9), CC15, CC45</td>
<td>lukED (12), lukPV (2) eta (6), eta (11), etd (3)</td>
<td>tst (3), sea (7), sed (4), sej (10)</td>
</tr>
<tr>
<td></td>
<td>agrIV (3)</td>
<td>CC5, CC45</td>
<td>lukED (3), lukPV eta, eta (2), etd</td>
<td>tst (2), sea (2), sej (3), ser (2)</td>
</tr>
<tr>
<td>V2a (5)</td>
<td>agrIV (5)</td>
<td>CC5 (4), CC45</td>
<td>lukED (4), lukPV (5) eta (5), eta (5), etd</td>
<td>tst (5), sea (5), sel (4)</td>
</tr>
<tr>
<td>V2b (16)</td>
<td>agrIII (16)</td>
<td>CC5 (4), CC30 (11)</td>
<td>lukED (16), lukPV (7) eta (6), eta (15)</td>
<td>tst (15), sea (15), sed, sej</td>
</tr>
<tr>
<td>V2c (34)</td>
<td>agrII (32)</td>
<td>CC5 (28), CC15, CC45</td>
<td>lukED (32), lukPV (5) eta (12), eta (28), etd (2)</td>
<td>tst (10), sea (13), sed (25)</td>
</tr>
<tr>
<td></td>
<td>agrIV</td>
<td>CC5</td>
<td>lukED, eta, etd</td>
<td>tst, sea, sec</td>
</tr>
<tr>
<td></td>
<td>agrI</td>
<td>CC45</td>
<td>lukED, eta, etd</td>
<td>etd</td>
</tr>
</tbody>
</table>

a The numbers of isolates (n) are listed in parentheses if the number is more than one.
b All isolates carried three to five hemolysin genes; exotoxin genes detected in all or most (more than 75%) isolates of the corresponding virulence cluster or subcluster are shown in boldface.

each of the HUCA isolates, independently of whether they were involved in spontaneous infections (13 up to 16), surgical wound infections (13 up to 21), pneumonia (13 up to 19), sepsis (11 up to 22), or SSSS (followed or not followed by sepsis, 14 and 18, respectively). Among the screened genes, only sep was significantly more frequent in isolates associated with sepsis (P = 0.032), and seb was more frequent in those that caused other infections (P = 0.032). Moreover, agrII and sep were more common in MRSA isolates than in MSSA isolates (P values of 0.029 and 0.048, respectively), while agrIII and tst were more common in MSSA isolates (P values of 0.021 and 0.032, respectively).

At a Jaccard coefficient of ca. 0.63, the dendrogram shown in Fig. 3 separated the HUCA isolates into two main virulence clusters (termed V1 and V2; Table 2). V1 incorporated all except one of the isolates that carried enterotoxin genes from plasmids (sed, seq, and/or ser), which were scattered among three of the four agr groups (agrII, agrI, and agrIV, with 12, 4, and 3 isolates, respectively). Most V2 isolates fell within one of three subclusters (V2a, V2b, and V2c). V2a included five agrIV isolates, all positive for lukPV, eta, etb, tst, sea, and the five hemolysin genes tested. With single exceptions, lukED, sec, and egc-like genes were also present. V2b grouped most of the agrIII isolates (16 out of 19) characterized by the occurrence of the five hemolysin genes, lukED (all isolates), etb, tst, sea (each with a single exception), and sec (two exceptions). In addition, most carried the egc-like cluster, and nearly half tested positive for lukPV. V2c included most of the agrII isolates which lacked SE genes from plasmids. The hemolysins genes, lukED, etb, sec, and egc were detected in all or most isolates of the subcluster (from ca. 80% up to 100%), while eta, tst, sea, seb, and sep were relatively common (32.4% to 44.1%). Finally, data compiled in Fig. 3 failed to reveal a correlation between virulence gene profile(s) and type of disease, with all clusters and subclusters grouping isolates involved in sepsis together with isolates that caused other types of infections. Accordingly, virulence factors apart from those screened in the present work, differential expression of the detected genes, and/or host-related factors may have been critical for the outcome of the disease (32, 35, 58).

DISCUSSION

Molecular epidemiology studies allowed the identification of the MRSA and MSSA CCs and clones that have been circulating in the HUCA during a recent time period. Overall, the frequency of MRSA isolates in this hospital (32.1%) coincided with that reported by the EPINE group, which surveys the prevalence of nosocomial infections in Spain (32.5% for the 2001-to-2003 period [2]), and was also in line with the results of a single-day surveillance study that analyzed 439 S. aureus isolates recovered from 143 Spanish hospitals during 2002 (30.5%) (5).

Most MRSA isolates from the HUCA (73%) were ST125-SCCmec IV isolates. This clone emerged in Spain during 1996, although it was first reported in 2001, and has now become predominant in Spanish hospitals (46, 47, 56). At a lower frequency, it was also found in Norway (13). As in the present study, a strong correlation between ST125 and spa type t067 was observed in both countries. Most of the remaining MRSA
isolates from the HUCA were also positive for SCCmec IV but belonged to other STs (i.e., ST5, ST228, or ST47); two carried SCCmec I and were of ST228, while one was not typeable by the applied method (60). The ST5-SCCmec IV (pediatric clone) (49) and ST228-SCCmec I (southern German clone) (48) clones are major hospital-acquired MRSA clones, widely distributed worldwide (7, 46, 56). In summary, typing of MRSA isolates from the HUCA revealed that nearly all belonged to CC5 (88.5%), one of the most diversified lineages of MRSA (48), and carried SCCmec IV (also 88.5%), specifically SCCmec IVc, which in Spain accounted only for 24% of the MRSA isolates, whereas subtype IVa was the most frequent (73.3%) (47).

As previously reported by other authors (7, 10, 52), a higher diversity of CCs was found among the MSSA isolates of the HUCA, which accounted for 67.9% of the analyzed isolates. Of them, 80.0% had a genetic background common to major MRSA lineages, namely, CC5 (38.2%), CC30 (25.5%), CC45 (14.5%), and CC8 (1.8%) (7, 17, 48). As was the case for MRSA, MSSA isolates with a CC5 genetic background mostly belonged to SmaI PFGE subclusters G1 and G2 and were of either ST5 or ST125; CC30 isolates fell into SmaI PFGE cluster B and were of ST30; and ST47 and ST45, both members of CC45, were separated by SmaI PFGE into clusters A and D. Four successful MSSA lineages different from the major MRSA clones, namely, CC12, CC15 (PFGE clusters F and E), CC51, and CC59 (each with one sporadic isolate), were also detected. Isolates of these four lineages have been observed in both nosocomial and community settings in different countries (1, 18, 19, 44, 54, 57). Finally, it is worth noting that, like in other studies (4, 20), a good concordance between typing by SmaI PFGE, spa type, and MLST type was found for the HUCA isolates. In fact, most isolates with the same MLST had similar SmaI profiles and, hence, grouped within the same PFGE cluster. However, several SmaI PFGE clusters and/or branches could be associated with a certain CC and even with a certain ST, consistent with the higher discrimination of the former method.

With regard to the virulence gene repertoires, the high number of exotoxin genes carried by each of the 81 isolates analyzed in the present work (from 11 up to 22) is remarkable. All HUCA isolates harbored genes encoding four or five hemolysins, one or two leukocidins, and five up to 13 enterotoxins. All tested positive for eae with or without sec, and most were positive for one or more genes encoding classical enterotoxins, including high percentages of sec and sea. Moreover, all except four isolates contained at least one exfoliatiin gene (eta, eth, and/or etd), whereas tst was present in half of them, and the leukotoxin gene lukPV was present in about one-fourth. In Spanish hospitals, the incidence of MSSA isolates carrying lukPV has increased from 0% in 2002 up to 36.4% in 2006 to 2007 (5, 47). In the present study, 66.7% of the lukPV-positive isolates were MSSA, but 33.3% were MRSA, and most belonged to the prevalent CC5. Of the 30 virulence genes screened, only lukM and see were absent from the series. Those that were detected appeared in many different combinations, with a total of 73 virulence profiles displayed by 81 isolates. A wide diversity of virulence gene combinations has also been reported for S. aureus isolates from England, Germany, and France (6, 22, 35).

Overall, the high number of exotoxins genes carried by each isolate and the rather indiscriminate distribution of these genes among different agr groups and genomic backgrounds, together with the striking diversity of exotoxin gene combinations, are consistent with a frequent acquisition and/or loss of the screened genes. It is widely accepted that mobile genetic elements carrying exotoxin genes can efficiently spread within lineages (35). In addition, they may be eventually capable of breaking interlineage barriers. An important barrier is the Saül type I restriction-modification system, which appears to be one of the major mechanisms underlying the clonal structure of S. aureus (11, 59). Nevertheless, a mobile genetic element can enter a CC from which it was originally excluded by means of a Saül restriction mutator. Once in such a mutant, the element might become modified at the specific sequences recognized by the hsdS1 and hsdS2 products characteristic of this particular lineage. Afterwards, its transfer to other members of the same lineage would be possible. In any case, the accumulation of exotoxin genes within S. aureus isolates is a matter of concern, since it may result in enhanced pathogenicity under appropriate circumstances (34). In the HUCA, the epidemiological surveillance of these potential “superbugs” could be facilitated, at least in the near future, by these findings with regard to the remarkable diversity of exotoxin gene combinations, which nearly differentiated at the strain level.

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