Prevalence and Evolution of Methicillin-Resistant *Staphylococcus aureus* in Spanish Hospitals between 1996 and 2002

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Pulsed-field gel electrophoretic analysis of 2,144 methicillin-resistant *Staphylococcus aureus* (MRSA) strains isolated from patients in Spanish hospitals over a 7-year period revealed 17 predominant profiles. Typing showed the replacement of Iberian clone E1 (ST247-MRSA-I) by two prevalent clones, E7 and E8, that are closely related to each other and have the same genetic background as ST125-MRSA-IV.

*Staphylococcus aureus* is a major cause of nosocomial infections, and methicillin-resistant *S. aureus* (MRSA) have spread around the world (30, 37). There are considerable variations in the prevalence of MRSA in different geographic areas. In Spain, the prevalence of MRSA has increased from 1.5% in 1986 to 31.2% in 2002 (5, 8).

In the 1980s, the first Spanish epidemic MRSA strain, called E1 in our pulsed-field gel electrophoresis (PFGE) scheme and known as the Iberian clone, spread throughout the country (1, 13). This clone presented two subtypes, E1a and E1b, and belonged to phage groups I and III at 100 RTD (routine test dilution) and phage group III at the RTD, respectively (3). The two subtypes presented only one-band difference in the restriction patterns by pulsed-field gel electrophoresis (PFGE) (41). This multiresistant clone has been isolated in other countries and accounted for a large proportion of MRSA isolates until 1995 (10, 11, 22, 23, 25).

To determine which clones are circulating in Spain and whether the strains had spread between hospitals, in this study we examined 2,144 MRSA isolates selected from all known MRSA strains and two MRSA strains representative of Iberian clone isolates during the beginning of the epidemic period (1989 to 1990). We studied the MRSA isolates by PFGE as previously described (25). The presence of the *mec* gene was confirmed by detection of the *mec* gene by multiplex PCR (18). The selected strains were from sporadic cases, and the strains were isolated from patients in 110 Spanish hospitals representing all Spanish Autonomous Communities. As initial markers, the phenotypic methods, phage typing and antibiotic profiling, were used to improve the selection of strains for further molecular epidemiology studies. For this reason, the screening was based on differences in: (i) the phage types obtained with the 23 phages of the Basic International Set at 100 RTD and 1,000 RTD and with the Specific Set of MRSA (3, 34, 40); (ii) the patterns of susceptibility to oxacillin, gentamicin, ciprofloxacin, rifampin, teicoplanin, and vancomycin, determined by the agar dilution method and interpreted according to CLSI (formerly NCCLS) guidelines (26); and (iii) the geographic location.

The most frequent phage types isolated from MRSA isolates were the nontypeable strains (60%), followed by groups III (20%) and I and III (8%). When the MRSA strains were retested at 1,000 RTD, the nontypeable percentage was reduced to 40%.

The antimicrobial resistance profiles showed a strong decrease in gentamicin-resistant (Gen r) strains from 60.8% in 1996 to 11% in 2001 and a subsequent increase in 2002 (24.2%). Gentamicin-sensitive (Gen s) strains emerged in Belgium (10) and France (14, 17, 19, 20). A decrease in rifampin-resistant strains was detected; 34.8% of the strains were rifampin-resistant strains in 1996, but the value fell to 5.2% in 2002. However, the high rates of ciprofloxacin-resistant strains at the beginning of the study (84.2%) continued to increase to 92%, while no vancomycin- and teicoplanin-resistant strains were detected.

We identified the 17 most common PFGE types (Fig. 1) (including several subtypes); each of the most common PFGE types was isolated with the same pattern 10 times in a year.

A dendrogram (Fig. 2) of all predominant genomic groups was constructed by using the Dice coefficient, and gel findings were interpreted on the basis of standard criteria (39). Four branches were identified. The E1 clone (Iberian clone) and three subtypes of E1 and the E4, E5, E6, and E9 clones grouped together into one branch. Profiles E15, E16, and E17 grouped in another branch. There is a third branch, which contains some of the predominant clones isolated recently (E3, E7, E8, E10, and E11). Finally, the rest of the profiles with low similarity values (E2, E12, E13, and E14) grouped in the fourth branch.

Before 1996, the Iberian clone (E1) was the predominant strain, but it decreased (40.1% to 11.4%) from 1996 to 1998.
FIG. 1. PFGE patterns of representative predominant MRSA clones identified from 2,144 nosocomial MRSA strains during a 7-year period (1996 to 2002). The clones, including Iberian clone (E1) subtypes (E1a to E1d), E5 (Brazilian clone), E7 subtypes (E7a to E7c), E8 subtypes (E8a and E8 b) and λ molecular markers are shown above the lanes.

FIG. 2. Dendrogram showing the genetic relationships among the 17 PFGE profiles identified from 2,144 Spanish MRSA strains and the SSCmec detected.
Clones E1 and E6 were the predominant clones, but over time E7 and E8 clones became the predominant clones. In 1996, E8 was isolated for the first time. The frequency of E8 increased from 2.4% to 40.0% from 1996 to 2000 and subsequently decreased to 26.5%. Unlike the other clones, the frequency of clone E7 continued to increase from 4.1% to 32.3%. The frequencies of the other clones remained the same from 1998 to 2002. The geographic distributions of all identified clones were examined, but no special geographic distribution was revealed.

For further epidemiological studies, a representative sample set of all MRSA strains studied were considered in order to determine the type of staphylococcal chromosomal cassette (SSCmec) according to the genotypes observed and year of isolation (31). Also, a representative sample set of the defined genotypes was characterized by multilocus sequence typing (MLST) (15) in order to determine the origin and evolution of the genotypes.

Table 1 and Fig. 2 show the distribution of the SSCmec complex and MLST types in correlation with the previous name given in order to identify correctly the MRSA clones circulating in Spain.

We found several clones that harbored SSCmec type I (E1, E6, E9, E15, E16, and E17), belonging to ST228 and exhibiting a multiresistant profile. This sequence type (ST) had been previously described in Italy (6).

SSCmec type II includes the E12 clone, with a pattern similar to that of EMRSA-16 (24) and the same allelic profile as ST36. The E12 clone was involved in outbreaks in England, and it had been reported to be a major clone in a Spanish hospital (32), but in our study, it represented only 1.8% of all MRSA strains analyzed.

The E4 (Brazilian clone) and E5 clones (33, 38) belonged to SSCmec type IIIA. Both clones display multiresistant profiles, but they are scarce in Spain. These clones clustered in the same lineage as ST239.

SSCmec type IV was initially found in community-acquired MRSA isolates (7, 12, 28, 36) and is one of the most frequently acquired elements in hospital MRSA infections in other countries (16, 21, 27, 29, 35). In our study, type IV was the predominant type found recently and was found in the eight clusters in Spain (Fig. 1).

SSCmec type IV was identified the first time in clone E3, which had been lysed by phage 95 (9), and it shares the same allelic profile as ST146 in E10 and E11 clones isolated in a hospital in Tenerife, Spain (32).

The E13 clone contains ST22, which is related to the epidemic EMRSA-15 (24), isolated in several countries, while the E2 clone has ST45, which was identified in several European countries (15).

ST125 was found in Spain for the first time in 2001 (32). However, in our study, we demonstrated that ST125 emerged during 1996, and at present, it is responsible for more than 50% of the infections caused by nosocomial MRSA strains in Spain. ST125-IV has diverse patterns and includes E7, E8, and E11 clones. This ST also displays different gentamicin susceptibilities and contains Gen° and Gen' strains. Some authors

<table>
<thead>
<tr>
<th>PFGE type</th>
<th>Frequency (%)</th>
<th>ST-MRSA-SSCcme</th>
<th>Allelic profilea</th>
<th>Current name</th>
<th>Geographic location</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1</td>
<td>6</td>
<td>ST247-MRSA-I</td>
<td>3-3-1-12-4-4-16</td>
<td>Iberian</td>
<td>Berlin, Germany</td>
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<tr>
<td>E2</td>
<td>0.6</td>
<td>ST45-MRSA-IV</td>
<td>10-14-8-6-10-3-2</td>
<td>Tenerife</td>
<td>Tenerife, Spain</td>
</tr>
<tr>
<td>E3</td>
<td>2</td>
<td>ST146-MRSA-IV</td>
<td>1-4-3-1-4-12-1-10</td>
<td>Brazilian</td>
<td>Brazil</td>
</tr>
<tr>
<td>E4</td>
<td>1.2</td>
<td>ST239-MRSA-III</td>
<td>2-3-1-4-1-4-3</td>
<td>Southern Germany</td>
<td></td>
</tr>
<tr>
<td>E5</td>
<td>1.7</td>
<td>ST228-MRSA-I</td>
<td>1-4-1-4-12-24-29</td>
<td>Norway; Tenerife, Spain</td>
<td></td>
</tr>
<tr>
<td>E6</td>
<td>11.5</td>
<td>ST125-MRSA-IV</td>
<td>1-4-1-4-12-1-54</td>
<td>United Kingdom</td>
<td></td>
</tr>
<tr>
<td>E9</td>
<td>1.8</td>
<td></td>
<td></td>
<td>United Kingdom</td>
<td></td>
</tr>
<tr>
<td>E15</td>
<td>1.1</td>
<td></td>
<td></td>
<td>United Kingdom</td>
<td></td>
</tr>
<tr>
<td>E16</td>
<td>1.9</td>
<td></td>
<td></td>
<td>United Kingdom</td>
<td></td>
</tr>
<tr>
<td>E17</td>
<td>2</td>
<td></td>
<td></td>
<td>United Kingdom</td>
<td></td>
</tr>
<tr>
<td>E7</td>
<td>27</td>
<td>ST125-MRSA-IV</td>
<td>1-4-1-4-12-1-54</td>
<td>Norway; Tenerife, Spain</td>
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</tr>
<tr>
<td>E8</td>
<td>31.6</td>
<td></td>
<td></td>
<td>United Kingdom</td>
<td></td>
</tr>
<tr>
<td>E11</td>
<td>3.3</td>
<td></td>
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<td>United Kingdom</td>
<td></td>
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<tr>
<td>E12</td>
<td>1.9</td>
<td>ST36-MRSA-II</td>
<td>2-2-2-2-2-3-2</td>
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<tr>
<td>E13</td>
<td>0.7</td>
<td>ST22-MRSA-IV</td>
<td>7-6-1-5-8-8-6</td>
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<td></td>
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<tr>
<td>E14</td>
<td>0.9</td>
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</table>

a The allelic profile shows the allele numbers of the seven housekeeping genes in the following order: arcC-aroE-glp-gmk-pta-tpi-yqiL.
support the hypothesis that Gen\textsuperscript{a} strains emerged from a Gen\textsuperscript{b} population (2, 4).

Moreover, other authors suggest that ST125 and ST146 were derived from ST5 (pediatric clone), because they differ from ST5 in only one allele. ST125 has a different sequence in \textit{yqiL} (acetyl coenzyme A acetyltransferase), and ST146 differs in \textit{aroE} (shikimate dehydrogenase) (32).

In conclusion, the use of PFGE in combination with SS\textit{CmeC} and MLST typing has identified the clones causing the majority of MRSA infections in Spain. The epidemiological background of characterized Spanish MRSA strains show an increased prevalence of SS\textit{CmeC} type IV and the dissemination of a relatively few clones. Moreover, we found in our retrospective study that ST125, which mainly includes E7 and E8 clones, emerged for the first time in 1996 and has been isolated more often since then. This ST is responsible for more than half of nosocomial MRSA infections, with a significant decrease in the level of gentamicin resistance.

REFERENCES


