Molecular Characterization of Methicillin-Resistant *Staphylococcus aureus* Isolates Collected in Asunción, Paraguay

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We characterized 34 methicillin-resistant *Staphylococcus aureus* strains isolated in Paraguay in 2005. The strains belonged to two clones. The major clone (sequence type 5 [ST5] or ST221, staphylococcal cassette chromosome mec [SCCmec] type I) was similar to the Cordobes/Chilean clone spreading through South America, and the minor clone (ST239 or ST889, spa type 1037, SCCmec type IIIA) was related to the Brazilian clone.

Population genetic studies of methicillin-resistant *Staphylococcus aureus* (MRSA) have identified five major clonal groups forming five clonal complexes (CCs) designated CC5, CC8, CC22, CC30 and CC45. Each CC is composed of MRSA isolates with related multilocus sequence types. Highly prevalent clones include the archaic clone (CC8), the Iberian clone (CC8), the Brazilian clone (CC8), the pediatric clone (CC5), and the New York/Japan clone (CC5) (4, 11). These clones have also been genetically characterized by their spa type (based on the polymorphic region of the protein A gene *spa*) and by their staphylococcal cassette chromosome mec (SCCmec) type (corresponding to one of the five chromosomal cassettes encoding methicillin resistance).

MRSA strains have spread among hospitals worldwide. Their prevalence differs widely among countries and from one hospital to another in the same country. The Sentry Antimicrobial Surveillance Program conducted several studies during 1997 and 1998 in Latin America (Argentina, Brazil, Chile, Colombia, Mexico, Uruguay, and Venezuela) and reported MRSA prevalence rates of 21% in bloodstream infections, 31% in skin and soft-tissue infections, and 50% in cases of pneumonia (1, 12). The MRSA clones spreading in South America belonged mainly to the Brazilian clone (in Argentina, Brazil, Chile, and Uruguay) between 1992 and 1998, to the pediatric clone (in Colombia) between 1996 and 1998 (11), and to the Cordobes/Chilean clone (in Chile and Argentina) between 1998 and 2002 (13, 15).

We collected 96 *S. aureus* isolates between April 2005 and October 2005 from 81 patients admitted to Hospital de Clínicas in Asunción, Paraguay. Forty-two out of the 96 isolates (43.7%) were resistant to methicillin. All MRSA isolates were associated with hospital-acquired infections. Of these MRSA, 34 isolates (one isolate per patient) were characterized by means of standard microbiological methods. Toxin gene content and the gene regulator (*agr*) allele group (groups 1 to 4) were determined by using multiplex PCR and primers described previously (7, 8). Pulsed-field gel electrophoresis (PFGE) patterns were determined as described previously (2) and were analyzed with GelCompar II software (Applied Maths, Sint-Martens-Latem, Belgium). A PFGE type was defined by a percentage similarity of >80%; subtypes corresponded to single-band variants. The 34 PFGE patterns corresponded to six PFGE types (AS1 to AS6) (Fig. 1), and AS5 harbored two subtypes. The major PFGE type, AS1, comprised 21 isolates.

For each PFGE type, representative isolates were characterized by their antibiotic resistance profile (Phoenix automated microbiology system; BD Biosciences) according to the CASFM 2007 recommendations (at http://www.sfm.asso.fr/), capsular serotype (16), SCCmec type (10), ST (www.mlst.net), and spa type (Ridom Staph Type software; Ridom GmbH, Wursburg, Germany) (http://spaserver.ridom.de/) (6, 9).

The 27 isolates with PFGE types AS1 to AS3 were considered to belong to the same MRSA clone, as they shared the following characteristics: (i) sequence type ST5 (or for one ST221, a single locus variant of ST5); (ii) spa type t149; (iii) *agr* allele 2; (iv) *egc*, *lukED*, and *hlgv* genes; (v) SCCmec type I; (vi) homogeneous resistance to methicillin; (vii) resistance to kanamycin, tobramycin, gentamicin, and ofloxacin; and (viii) capsular serotype 5. This major clone belonged to CC5 and was related to the New York/Japan clone or the pediatric clone, sharing ST5 and related *spa* types but with a different SCCmec type (Table 1). The major clone was even more closely related to the Cordobes/Chilean clone (Argentina), sharing SCCmec type I and ST5 and having related *spa* types (only one variation) (Table 1) (13, 14). Hence, the major MRSA clone detected in Hospital de Clínicas (Paraguay) corresponded to the Cordobes/Chilean clone. In Cordoba (Argentina), the Cordobes/Chilean clone started to replace...
the Brazilian clone in 1999 (14) and became predominant in 2001.

Isolates belonging to PFGE types AS5 and AS6 (six isolates) were also considered to belong to the same MRSA clone, as they shared the following characteristics: (i) sequence type ST239 (or for one isolate ST889, a single locus variant of ST239); (ii) spa type t037; (iii) agr allele 1; (iv) lukED and hlgv genes (five isolates) or hlgv and hlb genes (one isolate); (v) SCCmec type IIIA; (vi) homogeneous resistance to methicillin; (vii) resistance to kanamycin, tobramycin, and gentamicin; OFX, ofloxacin; RIF, rifampin; TEL, tetracycline; ERY, erythromycin; LIN, lincomycin; FF, fosfomycin; and SXT, cotrimoxazole. The presence (+) or absence (−) of toxin genes egc (enterotoxin gene cluster), hlgv (gamma-hemolysin variant genes), lukED (leukocidin lukE and lukD genes), and hlb (beta-hemolysin gene) is shown. The other toxin genes were negative for all the strains (see to see and seh, staphylococcal enterotoxin genes type A to E and H; hlg, gamma-hemolysin gene; lukSF-PV, Panton-Valentine leukocidin genes; eta and etb, exfoliatin toxin genes type A and B; and tst, toxin shock syndrome toxin 1 gene). CCs were assigned according to the MLST database (http://www.mlst.net). For the PFGE types, AS stands for Asunción, Paraguay.

Some of these MSSA strains also showed the capacity to

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**FIG. 1.** Dendrogram constructed from the schematic representation of the PFGE types of MRSA isolates included in this study together with their characteristics, such as drug resistance, CC, ST, spa type, SCCmec type, PFGE pattern, PFGE type, agr allele group, and toxin gene content. Drug resistance is indicated as follows: KTG, resistance to kanamycin, tobramycin, and gentamicin; OFX, ofloxacin; RIF, rifampin; TEL, tetracycline; ERY, erythromycin; LIN, lincomycin; FF, fosfomycin; and SXT, cotrimoxazole. The presence (+) or absence (−) of toxin genes egc (enterotoxin gene cluster), hlgv (gamma-hemolysin variant genes), lukED (leukocidin lukE and lukD genes), and hlb (beta-hemolysin gene) is shown. The other toxin genes were negative for all the strains (see to see and seh, staphylococcal enterotoxin genes type A to E and H; hlg, gamma-hemolysin gene; lukSF-PV, Panton-Valentine leukocidin genes; eta and etb, exfoliatin toxin genes type A and B; and tst, toxin shock syndrome toxin 1 gene). CCs were assigned according to the MLST database (http://www.mlst.net). For the PFGE types, AS stands for Asunción, Paraguay. nd, not determined.
acquire Panton-Valentin leukocidin genes (15); however, none of our MRSA strains belonging to the Cordobes/Chilean clone was positive for these genes.

REFERENCES


TABLE 1. Genotypic characterization of MRSA clones

<table>
<thead>
<tr>
<th>Clonal complex</th>
<th>MRSA clone</th>
<th>spa type</th>
<th>spa repeat*</th>
<th>ST</th>
<th>SCCmec</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC5 Major clone (Asunción, Paraguay)</td>
<td>t149</td>
<td>r260a30017/13r17/20/17/12r17/12r17/16</td>
<td>5</td>
<td>I</td>
<td></td>
</tr>
<tr>
<td>Cordobes/Chilean clone</td>
<td>t131</td>
<td>r260p09017/13r17/20/17/12r17/16</td>
<td>5</td>
<td>I</td>
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<tr>
<td>Pediatric clone (HDE288)</td>
<td>t002</td>
<td>r260p23/17/34/3r20/17/12r17/16</td>
<td>5</td>
<td>IV</td>
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<td>New York/Japan clone (BK2464)</td>
<td>t002</td>
<td>r260p23/17/34/3r20/17/12r17/16</td>
<td>5</td>
<td>II</td>
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</tr>
<tr>
<td>New clone (HT20030601)</td>
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<td>CC8 Minor clone (Asunción, Paraguay)</td>
<td>t037</td>
<td>r15/12/16r02/25/17r24</td>
<td>239</td>
<td>IIIA</td>
<td></td>
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<td>Brazilian clone (HU25)</td>
<td>t138</td>
<td>r08/1602/25/17r24</td>
<td>239</td>
<td>IIIA</td>
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<td>Hungarian clone (HU106)</td>
<td>t538</td>
<td>r15r12/16r02/25r16r02/25r16r02/25r17/12r24</td>
<td>239</td>
<td>III</td>
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<td>Iberian clone (PER34)</td>
<td>t501</td>
<td>r11r19/21/12r12/17/34r24r34r22r25</td>
<td>250</td>
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<td>r11r19/21/12r17/34r24r34r22r25</td>
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<td>250</td>
<td>I</td>
<td></td>
</tr>
</tbody>
</table>

* Underlined spa repeats are those of MRSA clones used for comparison. Bold spa repeats indicate variation in the spa repeat.

b Clones written in bold type were studied in this article.

c Underlined spa repeats are those of MRSA clones used for comparison. Bold spa repeats indicate variation in the spa repeat.

d See references 13 and 14.

e See reference 3.

f See reference 5.