Detection of a New Community Genotype Methicillin-Resistant Staphylococcus aureus Clone That Is Unrelated to the USA300 Clone and That Causes Pediatric Infections in Colombia

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Detection of a New Community Genotype Methicillin-Resistant 
*Staphylococcus aureus* Clone That Is Unrelated to the USA300 Clone and That Causes Pediatric Infections in Colombia

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The dissemination of a clone of community genotype methicillin-resistant *Staphylococcus aureus* (CG-MRSA) that is related to USA300 has been reported in Latin America. We recently detected isolates of a new clone of CG-MRSA (*spa* type t1635 and ACME-negative) that was genetically unrelated to the USA300 clone and that causes infections in children in Colombia. This finding indicates the appearance of a new clone of CG-MRSA in our region.

USA300 is the predominant clone of community genotype methicillin-resistant *Staphylococcus aureus* (CG-MRSA) in North America, and it has been reported in many other regions of the world, including in Latin America (1, 2). This clone usually (ST8-MRSA-IVa) harbors the *sek*, *seq*, *bsaB*, *lukF-PV*, and *lukS-PV* genes, which encode the staphylococcal enterotoxins K and Q, bacteriocin, and Panton-Valentine leukocidin (PVL), respectively. Additionally, the clone typically contains a type I *agr* operon, a type I008 *spa* gene, and the arginine catabolic mobile element (ACME). Since 2006, the spread of a CG-MRSA clone that is genetically related to the USA300 clone and that causes infections in adults and children has been reported in Colombia and several South American countries (2–4). This variant (ST8-MRSA-IVc) is PVL positive, harbors the *sek*, *seq*, and *bsaB* genes, and, unlike USA300, does not possess ACME. Recently, in a prospective multicenter study of MRSA infections in pediatric patients in Bogotá, Colombia, we detected isolates of a new clone of CG-MRSA that was genetically unrelated to USA300. The purpose of this study was to determine the genetic and molecular characteristics of the isolates belonging to this emerging clone.

All CG-MRSA (154 isolates with SCCmec type IV and/or *lukF-PV*, *lukS-PV*, *seq*, *sek*, or *bsaB*) isolated from patients with confirmed clinical infections were collected systematically from 15 hospitals between April 2009 to June 2011 in Bogotá, Colombia. Informed consent and approval by the institutional review boards of the participating hospitals were obtained. Molecular characterization of the isolates included PCR amplification of the genes *mec*, *mecA*, *lukF-PV*, *lukS-PV*, *spa*, *sek*, *seq*, *bsaB*, *gmk*, *arcC*, and *opp* (the last two were used as ACME markers) as well as SCCmec, *spa*, and *agr* typing (5, 6). The genetic relatedness between the isolates was established by multilocus sequence typing (MLST) and restriction of the *arcC* and *gmk* genes (7), and pulsed-field gel electrophoresis (PFGE) results were interpreted according to the criteria of Tenover and colleagues (8). The profile of susceptibility to 11 antibiotics was determined by the agar dilution method recommended by the Clinical and Laboratory Standards Institute (17).

Among 154 CG-MRSA isolates analyzed, 139 (90.3%) were genetically related to the USA300 clone, although they exhibited SCCmec IVc (3.1.2) and had no ACME, similar to clones previously reported in Colombia and in the Andean region (2, 3). However, 15 (9.7%) isolates exhibited PFGE pulsotypes unrelated to that of USA300 (>6 different bands and similarities of less than 80%). Of these, 8 (5.2%) isolates had closely related PFGE pulsotypes and type t1635 *spa* (YHGFMB0) (Fig. 1). Molecular characterization of these 8 isolates demonstrated that they contained SCCmec IVa and the genes *lukF-PV*, *lukS-PV*, *seq*, *sek*, *bsaB*, *sak* (prophage 3 specific), and group I *agr*. All isolates belong to clonal complex 8 (CC8). Representative isolates of the pulsotypes were analyzed by MLST, which identified two isolates as ST8 and two others as ST923. As in USA300, the *seq* and *sek* genes were found within pathogenicity island 5 (SaPI5), but unlike this clone, they did not contain ACME and had a 54-bp insertion in the *sausa300_0808* gene found in SaPI5. This insertion has been identified only in pathogenicity islands SaPIlov2 and SaI3 of strains ED133 (isolated from a sheep) and MW2, respectively. These results reveal the emergence of a CG-MRSA clone (CC8-MRSA-IVa; PVL positive and ACME negative) that, although it has some molecular features similar to USA300, is not genetically related to this clone. Susceptibility assays demonstrated that 8, 5, and 1 isolates were resistant to tetracycline (confirmed by the presence of the *tetK* gene), erythromycin, and gentamicin, respectively. One isolate exhibited multiple resistances to erythromycin, gentamicin, and tetracycline (Fig. 1). All of the isolates that were resistant to erythromycin exhibited an M phenotype.

The 8 isolates of the new clone were recovered from 6 pediatric patients (of various ages) and 2 newborns who had been treated at 6 of the 15 participating institutions, suggesting that these isolates were not associated with an epidemic event at one institution.
(Table 1 and Fig. 1). Six (75%) infections were classified as community onset MRSA (CO-MRSA; infection identified within the first 48 h of hospital admission) in children without healthcare-associated risk factors, whereas the remaining 2 (25%) cases were classified as hospital-onset MRSA infections (HO-MRSA, infection identified after 48 h of hospital admission) detected in two neonates who were treated at a single institution in June and July 2010, suggesting a common source and possible cross-transmission. No infections were detected in 2009, whereas in 2010 and 2011, 3 and 5 cases, respectively, were detected. These findings suggest an increased frequency of infections during the previous year, although the study was conducted only until June 2011. The clinical diagnosis of the patients included 5 skin and soft tissue infections (SSTI; 3 skin abscesses and 2 cases of abscessed cellulitis), 2 surgical-site infections (SSI) in the two newborns (one with deep SSI and another with organ space SSI that produced endocarditis), and 1 case of septic arthritis. Six patients were treated definitively with active antimicrobials for MRSA infections, and one was treated with cephalexin. Three patients (37.5%) required a PICU (pediatric intensive care unit) stay because of the severity of their infections; two had deep and organ space SSI, respectively, and the other was in a septic phase of septic arthritis. Five patients (62.5%) underwent incision and drainage, and full recovery was observed in all 8 cases.

Recently, the identification of isolates of S. aureus with spa type t1635 has been reported; some of these isolates exhibit molecular characteristics similar to those found in this study (9, 10). According to the reports on the Ridom Spa Server (http://spaserver.ridom.de/), the spa type t1635 has been found in European coun-

### Table 1: Characteristics of pediatric patients infected with the emergent CC8-MRSA-IVa PVL+/ACME− clone

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Case 1</th>
<th>Case 2</th>
<th>Case 3</th>
<th>Case 4</th>
<th>Case 5</th>
<th>Case 6</th>
<th>Case 7</th>
<th>Case 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case definition</td>
<td>HO-MRSA</td>
<td>HO-MRSA</td>
<td>CO-MRSA</td>
<td>CO-MRSA</td>
<td>CO-MRSA</td>
<td>CO-MRSA</td>
<td>CO-MRSA</td>
<td>CO-MRSA</td>
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<tr>
<td>Sex</td>
<td>Male</td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
<td>Female</td>
<td>Female</td>
</tr>
<tr>
<td>Age (years)</td>
<td>0.003</td>
<td>0.003</td>
<td>10.7</td>
<td>14.7</td>
<td>5.1</td>
<td>1.2</td>
<td>15.6</td>
<td>0.05</td>
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<tr>
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<td>Urban</td>
<td>Urban</td>
<td>Urban</td>
<td>Rural</td>
<td>Urban</td>
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<tr>
<td>Admission site</td>
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<td>NICU</td>
<td>ER</td>
<td>ER</td>
<td>ER</td>
<td>ER</td>
<td>HA</td>
<td>ER</td>
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<tr>
<td>Prior respiratory diseases</td>
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<td>None</td>
<td>None</td>
<td>None</td>
<td>Abscess</td>
<td>Abscess</td>
<td>None</td>
<td>None</td>
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<tr>
<td>Prior infectious diseases</td>
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<td>SSI</td>
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<td>Clinical diagnosis</td>
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<td>Abscess</td>
<td>SSTI</td>
<td>SSTI</td>
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<tr>
<td>Prior use of clinical devices</td>
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<td>Yes</td>
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<td>No</td>
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<td>No</td>
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<td>Hospital management</td>
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<td>NICU</td>
<td>HD</td>
<td>OXA-AMK</td>
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<td>OXA</td>
<td>PICU</td>
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<tr>
<td>Empirical antimicrobial therapy</td>
<td>VAN-TZP-GEN</td>
<td>AMP-AMK-GEN-TZP</td>
<td>OXA-GEN</td>
<td>CEF</td>
<td>OXA-LEX</td>
<td>OXA</td>
<td></td>
<td></td>
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<tr>
<td>Definitive antimicrobial therapy</td>
<td>VAN</td>
<td>VAN-SXT</td>
<td>SXT</td>
<td>CLI</td>
<td>CLI-AMK-ERY</td>
<td>LEX</td>
<td>VAN-RIF-SXT</td>
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<tr>
<td>Clinical outcome</td>
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<td>Improvement</td>
<td>Improvement</td>
<td>Improvement</td>
<td>Improvement</td>
<td>Improvement</td>
<td>Improvement</td>
<td></td>
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<tr>
<td>Duration of hospital stay (days)</td>
<td>58</td>
<td>17</td>
<td>6</td>
<td>6</td>
<td>9</td>
<td>4</td>
<td>6</td>
<td>27</td>
</tr>
</tbody>
</table>

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*a* HO-MRSA, hospital-onset MRSA infections (specimen isolated >48 h after hospital admission); CO-MRSA, community-onset MRSA infections (specimen isolated <48 h after hospital admission).

*b* NICU, neonatal intensive care unit; ER, emergency room; HA, hospital admission.

*c* COPD, chronic obstructive pulmonary disease.

*d* SSI, surgical-site infection organ space; SSSI, skin and soft tissue infection.

*e* PICU, pediatric intensive care unit; HD, hospitalization and drainage.

*f* SXT, trimethoprim-sulfamethoxazole; TZP; piperacillin-tazobactam; AMP, ampicillin; AMK, amikacin; CLI, clindamycin; CEF, cephalothin; ERY, erythromycin; LEX, cephalaxin; Rif, rifampin; OXA, oxacillin; GEN, gentamicin; VAN, vancomycin.
tries, such as Austria, France, Germany, Norway, Spain, Sweden, and Switzerland, but with a low circulation frequency. While analyzing infections caused by PVL+ S. aureus in Spain, Blanco and colleagues found two isolates with spa type t1635: one methicillin-susceptible S. aureus (MSSA) strain and one MRSA strain, the latter having molecular characteristics similar to those of the new clone detected (ST8-MRSA-IVa; PVL positive and ACME negative) and being genetically unrelated to USA300 (11). In Latin American countries, such as Colombia and Venezuela, some related isolates have been found; however, they were not identified as having a spa type, an agr group, or the insertion in the sausa300_0808 gene found in SaPI5, and those isolates show some differences in PFGE pulsotype (2, 12).

The possible emergence of this new clone of CG-MRSA that is causing infections in children of all ages in Bogotá could lead to temporal clonal replacement in the near future, as has happened before in Colombia and elsewhere, when new clones have emerged to replace existing ones. In the 1990s, the majority of MRSA infections in Colombia were hospital acquired and caused by the pediatric clone (ST5-MRSA-IV; PVL and ACME negative) (13). Subsequently, Cruz and colleagues reported the spread of a new dominant clone, the Chilean clone (ST5-MRSA-I; PVL and ACME negative), which has replaced the pediatric clone and circulates in Argentina, Chile, and Paraguay (14). The circulation of CG-MRSA isolates (ST8-MRSA-IVc) in Colombia was first reported in 2006 (4); subsequently, the frequency of these infections was approximately 25% of all MRSA infections in adults in 2008 (2, 15). Our group has recently determined that more than 95% of infections with MRSA in the pediatric population were caused by CG-MRSA isolates (16). These findings demonstrate the genetic success of the CG-MRSA clones in recent years, displacing the traditional hospital genotype MRSA clones (HG-MRSA; isolates with genetic characteristics traditionally found in strains isolated in hospital environments), as has begun to be reported in other countries (9). Possibly, this new emerging clone (CC8-MRSAIVA; PVL positive and ACME negative) and USA300 both originated from related MSSA isolates which acquired mobile genetic elements or single-nucleotide polymorphisms that thereafter differentiated the clones, because although the two clones exhibit low relatedness according to PFGE, they belong to the same clonal complex (CC8). Despite the small number of the novel isolates, given their similarities to USA300, the multiple-antibiotic-resistance phenotype raises the concern that they may have a significant temporal clonal replacement in the near future, as has happened before in countries (9). Possibly, this new emerging clone of CG-MRSA that is causing infections in children of all ages in Bogotá could lead to temporal clonal replacement in the near future, as has happened before in Colombia and elsewhere, when new clones have emerged to replace existing ones. In the 1990s, the majority of MRSA infections in Colombia were hospital acquired and caused by the pediatric clone (ST5-MRSA-IV; PVL and ACME negative) (13). Subsequently, Cruz and colleagues reported the spread of a new dominant clone, the Chilean clone (ST5-MRSA-I; PVL and ACME negative), which has replaced the pediatric clone and circulates in Argentina, Chile, and Paraguay (14). The circulation of CG-MRSA isolates (ST8-MRSA-IVc) in Colombia was first reported in 2006 (4); subsequently, the frequency of these infections was approximately 25% of all MRSA infections in adults in 2008 (2, 15). Our group has recently determined that more than 95% of infections with MRSA in the pediatric population were caused by CG-MRSA isolates (16). These findings demonstrate the genetic success of the CG-MRSA clones in recent years, displacing the traditional hospital genotype MRSA clones (HG-MRSA; isolates with genetic characteristics traditionally found in strains isolated in hospital environments), as has begun to be reported in other countries (9). Possibly, this new emerging clone (CC8-MRSAIVA; PVL positive and ACME negative) and USA300 both originated from related MSSA isolates which acquired mobile genetic elements or single-nucleotide polymorphisms that thereafter differentiated the clones, because although the two clones exhibit low relatedness according to PFGE, they belong to the same clonal complex (CC8). Despite the small number of the novel isolates, given their similarities to USA300, the multiple-antibiotic-resistance phenotype raises the concern that they may have a significant clinical impact in the event that they are easily transmissible. Furthermore, the emergence of new CG-MRSA clones could also be caused by the acquisition of SCCmec by various clones of S. aureus in the community, causing the clones that experience the least genetic cost in the acquisition of this element to predominate. It has been frequently observed that although many clones have the ability to acquire the same SCCmec, only a few predominate in their respective geographic niches (9), suggesting that there are specific genetic determinants for different regions.

The results of this study demonstrate the emergence of a new clone of MRSA with a community genotype and genetic characteristics that are different from those of USA300. This new clone, although still present at a low frequency (5.4%), may increase in circulation and disseminate. Therefore, it is necessary to continue surveillance studies, supported by molecular biology techniques, both in hospitals and in the community in order to monitor the behavior of infections caused by this distinct and newly emerging clone.

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REFERENCES


