Emergence and Dissemination of a Community-Associated Methicillin-Resistant Panton-Valentine Leucocidin-Positive Staphylococcus aureus Clone Sharing the Sequence Type 5 Lineage with the Most Prevalent Nosocomial Clone in the Same Region of Argentina

Claudia Sola,1 Hector A. Saka,1 the Cordoba MRSA Collaborative Study Group,† Ana Vindel,2 and José Luis Bocco1*

Departamento de Bioquímica Clínica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Centro de Investigaciones en Bioquímica Clínica e Inmunología (CIBICI-CONICET), Ciudad Universitaria, Pabellón Argentina, 5000 Córdoba, Argentina,1 and Laboratorio de Infecciones Nosocomiales, Instituto de Salud Carlos III, Centro Nacional de Microbiología, 28220 Majadahonda, Madrid, Spain2

Received 2 October 2007/Returned for modification 28 November 2007/Accepted 22 February 2008

Epidemiological surveillance for community-associated methicillin-resistant Staphylococcus aureus revealed prevalences of 33% and 13% in pediatric and adult patients, respectively, in Córdoba, Argentina, in 2005. This study describes for the first time the emergence and dissemination of the sequence type 5 (ST5) lineage as the most prevalent clone (89%) (pulsed-field gel electrophoresis type I-ST5-staphylococcal cassette chromosome type IVa-spa type 311) harboring the Panton-Valentine leukocidin and enterotoxin A genes.

Methicillin-resistant Staphylococcus aureus (MRSA) is a traditionally established nosocomial pathogen. Furthermore, virulent and nonmultiresistant community-associated MRSA (CA-MRSA) strains have been reported in many countries since 1993. CA-MRSA strains are commonly associated with skin and soft tissue infections, although invasive infections such as osteomyelitis and necrotizing pneumonia have been described. These strains frequently harbor staphylococcal cassette chromosome (SCCmec) type IV or V and the Panton-Valentine leukocidin (PVL) genes, along with a particular repertoire of virulence genes (4). Moreover, the simultaneous assessment of the genetic background, virulence gene profiles, and accessibility of virulence genes (agr) increases the discriminatory power of genetic investigations into S. aureus pathogenesis (4, 6).

In Argentina, MRSA is one of the most important pathogens causing health care-associated infections, with health care-associated MRSA (HA-MRSA) showing 48% prevalence in 2005 (15), whereas no data about CA-MRSA prevalence are available. The Cordobes/Chilean clone was reported as the most prevalent among HA-MRSA isolates (53% in 2001) in Córdoba, the second most populated city in Argentina (18). Additionally, one sporadic isolate that was susceptible to gentamicin, nonmultiresistant, and characterized as pulsed-field gel electrophoresis (PFGE) type I-sequence type 5 (ST5)-SCCmec type IVa was recovered in 2001 (18). Four other isolates with PFGE type II1 were also detected in another study carried out in 2004 (C. Sola and J. L. Bocco, unpublished data).

In this work, the prevalence of CA-MRSA infections was investigated during a surveillance period in 2005 in Córdoba. The molecular genetic characteristics and virulence gene contents of CA-MRSA strains, as well as the patients’ clinical features, were analyzed and compared to those for HA-MRSA strains isolated during the same time period. This prospective study of laboratory-based surveillance for MRSA infections in 14 hospitals (H1 to H14; 1,878 beds) in Córdoba, Argentina, was conducted in two steps: (i) single patient HA- and CA-MRSA isolates were collected during April to June 2005, and (ii) single patient CA-MRSA isolates were recovered during January to June 2006. Three tertiary-care community hospitals (H11 to H13) and a primary-care pediatric hospital (H14; 49 beds) were included in this study in addition to those previously reported (18). Since no HA-
MRSA was recovered in H14 in 2002 and since H14 had a high prevalence of CA-MRSA in 2005, all S. aureus strains, both MRSA and methicillin-susceptible S. aureus (MSSA), were analyzed to determine the genetic relationship between them during the 2006 period.

All isolates were identified by standard microbiologic procedures and were characterized by antibiotic susceptibility (2) and PFGE type (18). The mecA and pvl genes were determined for all strains by PCR (19). Representative isolates of CA-MRSA (all PFGE subtypes) and HA-MRSA (more prevalent subtypes) were characterized by multilocus sequence typing (MLST) and SCCmec and spaA typing, as previously described (18). The sequences obtained by spaA typing were compared to those held on the SpaServer (http://spaserver.ridom.de) (5). Representative isolates of the major PFGE types of MSSA recovered in H14 were also characterized by multilocus sequence typing and spaA typing. The agr group (1 to 4) (14) and virulence gene contents (4, 7, 14) were detected by PCR (Table 1).

A total of 376 isolates of S. aureus were collected during the 2005 period. The proportions for HA-MRSA and CA-MRSA

<table>
<thead>
<tr>
<th>Organism</th>
<th>PFGE type/n (%)</th>
<th>PFGE subtype/n (%)</th>
<th>ST/CC</th>
<th>RDOM spa type</th>
<th>spaA repeats</th>
<th>SCCmec type</th>
<th>pvlP</th>
<th>Virulence gene profilec</th>
<th>agr type</th>
<th>Non-β-lactam drug resistance (%)no</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA-MRSA</td>
<td>I4/2 (89)</td>
<td></td>
<td></td>
<td></td>
<td>311</td>
<td>TJM BDMG MK</td>
<td></td>
<td>IVa</td>
<td>+</td>
<td>eec-eglukDE</td>
</tr>
<tr>
<td></td>
<td>I1/34 (81)</td>
<td>5/5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>I1/1 (2)</td>
<td>5/5</td>
<td></td>
<td></td>
<td>2121</td>
<td>TJM BDMG MK</td>
<td></td>
<td>IVa</td>
<td>+</td>
<td>eec-eglukDE</td>
</tr>
<tr>
<td></td>
<td>I1/1 (2)</td>
<td>5/5</td>
<td></td>
<td></td>
<td>1125</td>
<td>TJM BDMG MK</td>
<td></td>
<td>IVc</td>
<td>+</td>
<td>eec-eglukDE</td>
</tr>
<tr>
<td></td>
<td>I2/2 (5)</td>
<td>5/5</td>
<td></td>
<td></td>
<td>1452</td>
<td>TJM BDMG MK</td>
<td></td>
<td>IVa</td>
<td>-</td>
<td>eec-eglukDE</td>
</tr>
<tr>
<td></td>
<td>I3/1 (5)</td>
<td>5/5</td>
<td></td>
<td></td>
<td>002</td>
<td>TJM BDMG MK</td>
<td></td>
<td>IVa</td>
<td>-</td>
<td>eec-eglukDE</td>
</tr>
<tr>
<td></td>
<td>I4/3 (7)</td>
<td>5/5</td>
<td></td>
<td></td>
<td>311</td>
<td>TJM BDMG MK</td>
<td></td>
<td>IVa</td>
<td>+</td>
<td>eec-eglukDE</td>
</tr>
<tr>
<td></td>
<td>C/3 (6)</td>
<td></td>
<td></td>
<td></td>
<td>002</td>
<td>TJM BDMG MK</td>
<td></td>
<td>IVNv</td>
<td>-</td>
<td>eec-eglukDE</td>
</tr>
<tr>
<td></td>
<td>C/1/3 (33)</td>
<td>100/5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>C/7/3 (33)</td>
<td>100/5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>C/9/1 (33)</td>
<td>100/5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>I4/3 (7)</td>
<td>5/5</td>
<td></td>
<td></td>
<td>311</td>
<td>TJM BDMG MK</td>
<td></td>
<td>IVa</td>
<td>+</td>
<td>eec-eglukDE</td>
</tr>
<tr>
<td></td>
<td>B/24 (18)</td>
<td></td>
<td></td>
<td></td>
<td>037</td>
<td>WGA KAMQ M</td>
<td>III</td>
<td>-</td>
<td>lukDE-bsa</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>B/16 (25)</td>
<td>239/8</td>
<td></td>
<td></td>
<td>037</td>
<td>WGA KAMQ M</td>
<td>III</td>
<td>-</td>
<td>lukDE-bsa</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>B/29 (36)</td>
<td>239/8</td>
<td></td>
<td></td>
<td>037</td>
<td>WGA KAMQ M</td>
<td>III</td>
<td>-</td>
<td>lukDE-bsa</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>C/20 (15)</td>
<td></td>
<td></td>
<td></td>
<td>002</td>
<td>TJM BDMG MK</td>
<td></td>
<td>IVNv</td>
<td>-</td>
<td>eec-eglukDE</td>
</tr>
<tr>
<td></td>
<td>C/1/6 (43)</td>
<td>100/5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>C/15/5 (21)</td>
<td>100/5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>M/1 (10)</td>
<td></td>
<td></td>
<td></td>
<td>002</td>
<td>TJM BDMG MK</td>
<td></td>
<td>+</td>
<td>eec-eglukDE</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>M/1/1 (50)</td>
<td>5/5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>U/2 (20)</td>
<td></td>
<td></td>
<td></td>
<td>08/8</td>
<td>ND</td>
<td>ND</td>
<td></td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>U/21 (50)</td>
<td>8/8</td>
<td></td>
<td></td>
<td>08/8</td>
<td>ND</td>
<td>ND</td>
<td></td>
<td>-</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>N/1 (10)</td>
<td></td>
<td></td>
<td></td>
<td>012</td>
<td>WGA KAMQ M</td>
<td>ND</td>
<td></td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>T/1 (10)</td>
<td></td>
<td></td>
<td></td>
<td>012</td>
<td>WGA KAMQ M</td>
<td>ND</td>
<td></td>
<td>-</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>V/1 (10)</td>
<td></td>
<td></td>
<td></td>
<td>012</td>
<td>WGA KAMQ M</td>
<td>ND</td>
<td></td>
<td>-</td>
<td>ND</td>
</tr>
</tbody>
</table>

a CA-MRSA, n = 47 (22 in 2005 and 25 in 2006); HA-MRSA, n = 134 (2005 period); CA-MSSA, n = 10 (2006 period).

b lukS-PV–lukF-PV.

c The following genes were analyzed, and those detected are indicated: for enterotoxins, cluster eeg (seg, sei, sem, sen, and seo), sea, seb, sec, sed, see, seh, sej, and sek; for toxic shock syndrome toxin 1, tst-1; for exfoliative toxin, eta and elt; for leukocidin, lukED and the class F leukocidin lukM; for adherence, cna and bap; and for bacteriocin, bsa.

d Resistance to the non-β-lactams gentamicin (GEN), ciprofloxacin (CIP), erythromycin (ERY), clindamycin (CLIc and CLIi); constitutive and inducible resistance to macrolides-lincosamides-streptogramin B, respectively), rifampin (RIF), chloramphenicol (CHL), trimethoprim-sulfamethoxazole (SXT), and minocycline (MIN) was determined. The percentage of strains resistant to these antibiotics within each PFGE type is indicated when more than one isolate with this PFGE type was detected.

e ND, not determined.
TABLE 2. Demographic and clinical characteristics of patients with CA-MRSA versus HA-MRSA infections during April through June 2005a

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>HA-MRSA (n = 134)</th>
<th>CA-MRSA (n = 22)</th>
<th>P valueb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>48 (36)</td>
<td>11 (50)</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>86 (64)</td>
<td>11 (50)</td>
<td>0.20</td>
</tr>
<tr>
<td>Age group (yr)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Children (0–14)</td>
<td>16 (12)</td>
<td>7 (32)</td>
<td>0.02</td>
</tr>
<tr>
<td>Young adults (&lt;30)</td>
<td>8 (6)</td>
<td>13 (59)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Children and young adults</td>
<td>24 (18)</td>
<td>20 (91)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Adults (&gt;30)</td>
<td>93 (69)</td>
<td>2 (9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Elderly (&gt;69)</td>
<td>17 (13)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infection type</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skin and soft tissue</td>
<td>37 (28)</td>
<td>20 (90)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Abscesses</td>
<td>9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cellulitis</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Folliculitis</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Foruncles</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wound</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surgical site</td>
<td>37</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bloodstream</td>
<td>37 (28)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sepsis</td>
<td>32</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Catheter associated</td>
<td>32</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bone and joint</td>
<td>15 (11)</td>
<td>1 (5)</td>
<td>0.3</td>
</tr>
<tr>
<td>Osteomyelitis</td>
<td>6</td>
<td>1c</td>
<td></td>
</tr>
<tr>
<td>Arthritis</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prosthetic joint</td>
<td>7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Respiratory tract</td>
<td>23 (17)</td>
<td>1 (5)</td>
<td>0.1</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ventilator-associated pneumonia</td>
<td>18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute middle otitis</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urinary tract</td>
<td>9 (8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>13 (10)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a CA-MRSA was defined as MRSA that was isolated from patients who acquired it outside the hospital setting or within 48 h of admission and had no direct or indirect exposure to the health care system in the previous year. HA-MRSA infection was defined according to published criteria, including infection diagnosed in patients with risk factors for hospital acquisition during the previous 6 months (17, 18).
b P values are based on the chi-square test or Fisher’s exact test if 20% of the expected values were smaller than 5 for comparisons of CA-MRSA versus HA-MRSA for each of the categorical variables (males, females, age group, and infection site). A P value of <0.05 was considered statistically significant with chi-square distribution. Epi Info version 6.0.4 software (Centers for Disease Control and Prevention, Atlanta, GA) was used.
c The patient affected by CA-MRSA osteomyelitis also had metastatic pulmonary disease and severe sepsis syndrome.

Infections were 57% (134 MRSA/235 HA S. aureus isolates) and 16% (22 MRSA/141 CA S. aureus isolates), respectively. The prevalence of CA-MRSA differed significantly between pediatric and adult patients (33% [7 CA-MRSA/21 CA S. aureus isolates] versus 13% [15 CA-MRSA/120 CA S. aureus isolates]; P = 0.02). Twenty-five episodes of CA-MRSA infections were detected during the 2006 period. In H14, 11 CA-MRSA and 10 CA-MSSA isolates were recovered (52% prevalence). These results revealed the emergence of CA-MRSA in the community; moreover, this is the first report of the prevalence of CA-MRSA in Argentina.

Different definitions for distinguishing CA-MRSA and HA-MRSA as well as differences in the settings and populations analyzed could explain why the prevalence rates of CA-MRSA reported so far vary widely among different studies and countries. The high prevalence (31% in 2005 and 52% in 2006) observed in H14 could reflect the true prevalence of CA-MRSA in this region, because the patients in this hospital were pediatric outpatients affected by nonsevere infections. In fact, patients with mild S. aureus skin diseases are often treated empirically and are more frequently treated in primary or intermediate-care institutions (such as H14) than in tertiary-care community hospitals (such as H11 to H13). In conclusion, the results from H14 during 2005 and 2006 strongly suggest that the current prevalence of CA-MRSA in Argentina may be higher than was detected in all hospitals in 2005 and thus may call for closer systematic surveillance.

The CA-MRSA strains have been shown to be continent specific for some STs, although the results of a recent study suggest intercontinental exchanges of these PVL-positive clones and detection of new ones since 2003. Among the latter, ST5 was described only for sporadic isolates from Europe and the United States (1, 10, 11, 16, 20). Nevertheless, in this study, among all the CA-MRSA isolates, a predominant (89% [91% in 2005 and 88% in 2006]) major PFGE type, PFGE type I, was detected (Table 1), belonging to the ST5 lineage. All isolates of this PFGE type were characterized as agr type 2 and carried the IVa SCCmec type, except for one isolate (SCCmec type IVC). Most of them had spa type 311 (88%) and harbor pvl and sea genes (94%) (Table 1). Therefore, the emergence of the ST5 lineage in 2001 (18) and its dissemination as the most
prevalent clone among CA-MRSA is described for the first time in this study. Muller-Premru and coworkers reported isolates of the ST5 lineage harboring pvl genes but associated with SCCmec type I-spa type 002 instead of SCCmec type IVa-spa type 311 (12). The acquisition of pvl genes by this lineage is of extreme epidemiological importance for two reasons: (i) a high epidemic potential of the New York/Japan/ST5-II and Pediatric/ST5-IV pandemic clones was demonstrated in hospital settings, and (ii) the lineage has the ability to acquire vancomycin resistance (18).

In addition, two STs (ST917/CC8 and ST918 [singleton]) were described in this study for the first time, along with the already-characterized ST100 (three isolates), among CA-MRSA strains (Table 1).

The distributions of CA-MRSA and HA-MRSA with respect to patients' clinical features, age, and sex are shown in the Table 2. In agreement with several reports (13), the results of this work showed that most CA-MRSA isolates were from skin and soft tissue infections and occurred with higher frequency among children and young adults (Table 2). Invasive and severe infections (three cases), such as sepsis with pulmonary involvement and bone and joint infections, were also detected. This supports the high virulence of the new CA-MRSA clone detected in Argentina. The presence of PVL appears to be associated with increased severity (9), and the sea genes were linked to severe and invasive S. aureus infections (3). Thus, the presence of both pvl and sea genes, as observed for the clone described here, seems to be a genetic signature of highly toxic/superantigenic strains involved in CA-MRSA infections.

It is of significance that CA-MRSA strains have been reported as the cause of HA-MRSA infections (8). In our study, four patients from different hospitals with surgical site infections were considered to have CA-MRSA infections according to the molecular characterization. Hence, the systematic follow-up of infections caused by CA-MRSA and HA-MRSA in this region is necessary to determine whether this new virulent CA-MRSA clone could also emerge as a cause of HA-MRSA infections. The eventual spread of this CA-MRSA clone in hospitals and in the community could be a serious public health challenge in the coming years.

On the other hand, among the HA-MRSA strains, the Cordobes/Chilean clone is still the most prevalent (64% in 2005) (18) since 1999, and it behaves at present like an endemic clone. Although the total number of HA S. aureus infections did not vary significantly (data not shown), a significant increase in the prevalence of HA-MRSA infections was observed in 2005 (43% in 2001 versus 57% in 2005) (P < 0.0001). Thus, this clone not only gained this dominant behavior at the expense of the other epidemic strains (e.g., the Brazilian clone) but also enhanced the burden of HA-MRSA infections.

The ST30 and ST5 lineages were the most prevalent among invasive MSSA strains with the capacity to acquire pvl genes in nosocomial and community settings in this region during 1999 to 2002 (19). In this study, most strains of MRSA and MSSA belonged to the same PFGE type I and had similar molecular characteristics (Table 1 and Fig. 1A and B). Comparative analysis of virulence gene content and genetic background among those local strains along with the sequential emergence of different genotypes of PFGE type I (Fig. 1C) allowed us to delineate the possible evolutionary pathway within clonal complex 5 (CC5) (Fig. 1D). Therefore, the new epidemic CA-MRSA clone likely arose from insertion of SCCmec type IVa into a successful pvl- and sea-positive MSSA clone (Fig. 1B and D). The type diversity of the SCCmec elements (types IVa, IVc, IVNv, and I) identified in ST5/ST100-CC5 indicates that their integration occurred independently in different time periods. This observation argues in favor of the existence of a successful MSSA clone having a genomic background (PFGE type M-ST5-spa type 002) with the capacity to acquire pvl genes, adapted to the local ecological niche, and more receptive to different SCCmec types. These characteristics could confer a selective advantage for its dissemination in Argentina. According to these results, it is interesting to hypothesize that both the prevalent CA-MRSA and HA-MRSA clones could have evolved from the same successful MSSA ancestor (Fig. 1D).

Our results reveal that PVL-producing MRSA strains must now be considered as a possible cause of CA infections in Argentina when choosing therapeutic options. Most CA-MRSA strains were resistant only to β-lactam antibiotics and were susceptible to mupirocin and fusidic acid (only 10% resistance to clindamycin was detected).

In conclusion, the emergence of CA-MRSA in Cordoba, Argentina, was due to the expansion, for the first time, of a virulent clone belonging to the ST5 lineage associated with an SCCmec IVa element and thus differing from the ST30-IVc clone disseminated in the bordering country of Uruguay since 2003.

This work was supported by the National Council for Scientific Research and Technology of Argentina (CONICET), Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT) (grant PICT 05-13446 to J.L.B.), Secretaría de Ciencia y Técnica-Universidad Nacional de Córdoba (SECyT-UNC), and Agencia Córdoba Ciencia. C.S. is a fellow recipient of the SECyT-UNC. C.S. and J.L.B. are career investigator members of CONICET.

REFERENCES


