Spread of Epidemic MRSA-ST5-IV Clone Encoding PVL as a Major Cause of Community Onset Staphylococcal Infections in Argentinean Children

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Abstract

Background: Community-associated methicillin-resistant Staphylococcus aureus-(CA-MRSA) strains have emerged in Argentina. We investigated the clinical and molecular evolution of community-onset MRSA infections (CO-MRSA) in children of Córdoba, Argentina, 2005–2008. Additionally, data from 2007 were compared with the epidemiology of these infections in other regions of the country.

Methodology/Principal Findings: Two datasets were used: i) lab-based prospective surveillance of CA-MRSA isolates from 3 Córdoba pediatric hospitals-(CBH1-H3) in 2007–2008 (compared to previously published data of 2005) and ii) a sampling of CO-MRSA from a study involving both, healthcare-associated community-onset-(HACO) infections in children with risk-factors for healthcare-associated infections-(HRFs), and CA-MRSA infections in patients without HRFs detected in multiple centers of Argentina in 2007. Molecular typing was performed on the CA-MRSA-(n: 99) isolates from the CBH1-H3-dataset and on the HACO-MRSA-(n: 51) and CA-MRSA-(n: 213) isolates from other regions. Between 2005–2008, the annual proportion of CA-MRSA/CA-S. aureus in Córdoba hospitals increased from 25% to 49%, P<0.01. Total CA-MRSA infections increased 3.6 fold-(5.1 to 18.6 cases/100,000 annual-visits, P<0.0001), associated with an important increase of invasive CA-MRSA infections-(8.5 fold). In all regions analyzed, a single genotype prevailed in both CA-MRSA (82%) and HACO-MRSA(57%), which showed pulsed-field gel electrophoresis-(PFGE)-type-”I”, sequence-type-5-(ST5), SCCmec-type-Iv, spa-t311, and was positive for PVL. The second clone, pulsortype-N/ST30/CC30/SCCmecIVc/t019/PVL*, accounted for 11.5% of total CA-MRSA infections. Importantly, the first 4 isolates of Argentina belonging to South American-USA300 clone-(USA300/ST8/CC8/SCCmecIVc/t008/PVL+/ACME) were detected. We also demonstrated that a HA-MRSA clone-(pulsotype-C/ST100/CC5) caused 2% and 10% of CA-MRSA and HACO-MRSA infections respectively and was associated with a SCCmec type closely related to SCCmecIV(2B6S).

Conclusions/Significance: The dissemination of epidemic MRSA clone, ST5-IV-PVL* was the main cause of increasing staphylococcal community-onset infections in Argentinean children (2003–2008), conversely to other countries. The predominance of this clone, which has capacity to express the h-VISA phenotype, in healthcare-associated community-onset cases suggests that it has infiltrated into hospital-settings.

Introduction

Methicillin resistant Staphylococcus aureus (MRSA) infection has traditionally been associated with healthcare settings (HA-MRSA) until its emergence in the community during late 1990’s. This global phenomenon was caused by strains of community-associated methicillin-resistant Staphylococcus aureus (CA-MRSA) that emerged from some major genetic lineages, commonly designated by their multilocus sequence type (MLST) or by their pulsed field gel electrophoresis (PFGE) pattern (ST1, ST8-USA300, ST30, ST39, ST93 and ST80), with a specific geographical pattern. These strains are frequently non-multiresistant to antibiotics; most of them harbor the staphylococcal cassette chromosome mec (SCCmec) type IV(2B) or V(3C2) and the

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Panton-Valentine leukocidin (PVL) genes [1–3]. Purulent skin and soft tissue infections are the most common clinical manifestations of CA-MRSA, although increasing reports of serious infections in children with high mortality rates have raised concern during recent years [4,5]. Vancomycin has been the mainstay of treatment for this type of infections and the emergence of resistance is worrying [6,7]. Importantly, CA-MRSA strains have entered the healthcare settings; hence, in some countries, strains of both, CA-MRSA and HA-MRSA circulate in hospitals and communities, causing infections of healthcare or community onset (HO and CO, respectively) [8–10].

In Latin America, three PVL-positive epidemic CA-MRSA clones have been described: i) MRSA ST30-IV clone [11], ii) MRSA ST5-IV clone [12,13] and iii) MRSA ST8-IV clone [14]. The first two were detected in southern areas of South America and the third one in the North.

Epidemiological surveillance for MRSA in Córdoba, Argentina, revealed the emergence of ST5-IV-PVL+ genotype as the most prevalent (89%) CA-MRSA clone in 2005. This clone had displaced in the community setting an “escaped” HA-MRSA clone, which was non-multiresistant to antibiotics, lacked the pvl genes and was characterized as ST100 associated with a SCCmec related to IVc [12,15]. Prospective surveillance of community onset S. aureus infections in children from Argentina during 2007, detected a prevalence of 62% (281/447 CA-S. aureus) of CA-MRSA infections; 38% of which were invasive infections [16].

A paucity of data about molecular epidemiology of CA-MRSA infections from South America is a current problem and therefore, it is difficult to follow evolving trends [17–19].

Herein, we report the molecular evolution and antimicrobial resistance profiles of MRSA strains in the community along with the clinical characteristics of these infections in children of Córdoba-Argentina between 2003 and 2008.

We also compared the clinical and molecular epidemiology of CO-MRSA infections in children with risk factors for healthcare-associated infections (HRFs), (healthcare-associated, community-onset infections [HACO]), and in patients without HRFs (CA-MRSA infections), from multiple centers of central, eastern and northern regions of Argentina during 2007.

Materials and Methods

Ethics statement

This study was reviewed and approved by the institutional Ethical Review Board of each Hospital: Hospital de Pediatría Prof. Dr. Juan P. Garrahan; Hospital General de Niños Dr. Pedro de Elizalde; Hospital de Niños Dr. O. Alasia, Santa Fe; Hospital de Niños de San Justo; Hospital Juan Pablo II, Corrientes; Hospital de Niños J. Vilela, Santa Fe; Hospital Pediátrico Dr. A.L. Castelán, Chaco; Hospital Materno Infantil de Mar del Plata; Don Victorio Tetamanti, Hospital de Niños de la Santísima Trinidad de Córdoba, Hospital Infantil Municipal de Córdoba; Hospital Pediátrico del Niño Jesús de Córdoba. Written informed consent was obtained from each child’s parents for review of the medical information and characterizations of the responsible isolates. However, for the aims of this study, data were processed anonymously. Additionally, the authors declare that all clinical investigation has been conducted according to the principles expressed in the Declaration of Helsinki.

Study design and case definitions

Longitudinal study, Córdoba, 2003–2008. To analyze the molecular evolution and clinical characteristics of CA-MRSA infections in children (≤18 years of age) in Córdoba, all consecutive single patient isolates (n: 99) identified as CA-MRSA according to CDC criteria (see definitions) by a laboratory-based surveillance program of S. aureus infections during 2007–2008 from three children’s hospitals (CBAH1, CBAH2 and CBAH3) were prospectively included.

These molecular data were compared with those obtained in 2005 [12]. In that year, all S. aureus infections were identified by the Clinical Microbiology Laboratory of each hospital (CBAH1, CBAH2 and CBAH3) and the medical records were reviewed for epidemiologic classification (see definitions).

Additionally, 22 CA-MRSA isolates prospectively collected in CBAH1 between 2003 and 2006 following the CDC criteria (see definition) were analysed as well, along with those detected for this study (2007–2008).

Transversal study, Argentina, 2007. To examine the clinical and molecular epidemiology of CA-MRSA and HACO-MRSA infections among children from central, northern and eastern regions of Argentina in 2007, random samples representative of each hospital participating in the prospective surveillance of community onset S. aureus infections in children from Argentina (CSACHARG) were analyzed. The clinical features, frequency and outcome of CA-MRSA infections identified in that study have already been published [16]. Briefly, a total of 840 S. aureus infections were diagnosed during 2007, 582 of them were of community onset; among them, 135 children with HRFs were excluded from that study (75 infected with HACO-MRSA and 60 with HACO-MSSA). Of the remaining 447 isolates, 281 (62%) were CA-MRSA and 160 CA-MSSA. The following sample was selected for this study: CA-MRSA isolates: 213 of 281 (Buenos Aires city: 101, Buenos Aires province: 11, Corrientes: 22, Chaco: 19 and Santa Fe: 60). HACO-MRSA isolates: 51 of 75 (Buenos Aires city: 25, Buenos Aires province: 5, Corrientes: 5, Chaco: 5 and Santa Fe: 11).

For each case of S. aureus isolated from community onset infections from both collections (CBAH1-H3 and CSACHARG), the relevant medical information was examined, including diagnosis, site and clinical details of infection, treatment information, demographic data (age, gender), underlying illnesses (chronic conditions) [8,20] and the presence of the following risk factors for healthcare-associated infections: existence of an invasive device (e.g., vascular catheter, G-tube); any history of MRSA infection or colonization, surgery, hospitalization, dialysis, or residence in a long-term care facility within the 12 months prior to the culture [21–23].

Definitions

Community-associated methicillin resistant S. aureus (CA-MRSA) infections were defined as cases from patients without HRFs during the previous year, who acquired the infection outside hospital settings or within 48 h of admission, according to the CDC criteria [22].

Healthcare-associated infections (HA-MRSA) were defined using previously published definitions [21,22], which include: i) the classical nosocomial or hospital-onset infection (HO-MRSA), defined as a those occurring >48 h after hospital admission, not present on admission (no cases of this type were analyzed in this study) and ii) Healthcare-associated community-onset (HACO-MRSA) infections: those occurring in patients with HRFs, having a positive culture within ≤48 h after hospital admission. Hence, Community-onset methicillin resistant S. aureus (CO-MRSA) infections include both the CA-MRSA and HACO-MRSA infections.

Invasive infections were defined by one or more of the following conditions: bacteremia, endocarditis, pneumonia, pleural abscess, lymphadenitis, septic arthritis, osteomyelitis, necrotizing fasciitis, pyomyositis or another illness in which S. aureus was isolated from
normally sterile body fluids [20,21]. Sepsis was defined on the basis of the systemic inflammatory response syndrome criteria [24]. Skin diseases (skin and soft tissue, SSTI) were defined as a primary skin infection such as an abscess, cellulitis, folliculitis or a skin infection spreading to contiguous tissues.

Surgical site infections (SSI) were not considered as skin diseases.

**Bacterial isolates and antimicrobial susceptibility**

*Staphylococcus aureus* were identified by standard microbiologic procedures. Antimicrobial susceptibility testing was performed by disk diffusion method [25]. A disc approximation test (D test) was used to detect inducible clindamycin resistance [25]. Screening for inducible clindamycin resistance was performed on all CO-MRSA isolates. Vancomycin and teicoplanin are used to detect inducible clindamycin resistance [25]. Screening for inducible clindamycin resistance was performed on all CO-MRSA isolates. Vancomycin and teicoplanin minimum inhibitory concentrations (MICs) were determined by agar dilution method [25]. A disc approximation test (D test) was performed on all CO-MRSA isolates. Vancomycin and teicoplanin minimum inhibitory concentrations (MICs) were determined by agar dilution method [25]. In addition, all MRSA isolates with a MIC of 1 or 2 µg/ml were further screened by 2 additional agar methods: MHA5T-Mueller-Hinton-agar and BHI4V-brain-heart-infusion-agar (5 µg/ml teicoplanin and 4 µg/ml vancomycin, respectively) [6,26,27]. MRSA isolates that were positive in at least one screening test were additionally tested by both, the Etest Macromethod (MET) and Etest glycopeptide-resistance detection strip-GRD E-test strips, (bioMérieux-CD) [6,26]. The strains h-VISA-Mu3 and h-VISA-Mu50 and the vancomycin-susceptible S. aureus strain ATCC 29213 were tested for all methods in parallel as positive and negative controls, respectively.

**Molecular typing and detection of genes associated with virulence**

In all CO-MRSA isolates, the PFGE of *Sma*I digests of chromosomal DNA were performed and interpreted as previously described [12]. *Sma*I restriction patterns (PFGE) were digitized, presented schematically and analyzed to calculate the Dice coefficient of correlation and to generate a dendrogram by the unweighted-pair group method using average linkage clusterings. Isolates differing by up to six fragments were considered to be subtypes of a given clonal type, and the similarity cut-off was 80%.

All CO-MRSA isolates were screened by PCR for accessory gene regulator (agr) type and for 22 specific staphylococcal virulence genes, as described elsewhere [12 and reference therein], comprising sequences specific for enterotoxins: sea, seb, sec, sed, sef, seq, seh, set, sej, sen, seo, srt, sem and seh; toxic shock syndrome toxin 1(TSST-1); tst; exfoliative toxins: eta and etb; PVL genes: lukS-PV-lukF-PV; leukocidin: lukED-lukD and the class F leukocidin: lukM; bacteriocin (bca), adhesins for collagen (cna) and for bone sialoprotein-binding protein (bhp). Detection of *arcA* gene, an indicator of the arginine catabolic mobile element (ACME), was previously determined sequence of strain USA300_FPR3757 (accession NC_007793). The primer sequences are as follows: arcA-F 5’-TCT ATT ACT GAG CCA GAA GTA CG-3’ and arcA-R 5’-CAC GTA ACT TGC TAG AAC GAG TA-3’ whose product has 733 bp expected size. USA300-0114 was used as reference strain for the USA300 clone.

Representative isolates of the most prevalent subtype (defined by PFGE) from all CO-MRSA strains were also characterized by multilocus sequence typing and spa typing [29,30].

**SCCmec typing**

The SCCmec types (I–VI) were evaluated for all CO-MRSA isolates by multiplex PCR [31] and by allotyping by conventional PCR [32]. SCCmec type IV was further sub-typed using published primers [33]. Furthermore, the assignment to the class B mec complex was confirmed by PCR with primers specific for *mecA* and *mecR1* [34] and in order to differentiate the types of SCCmec IV and VI, the *ccdB* typing was completed by PCR detection with primers specific for *ccdB* allotype 4 [34].

To investigate the presence of SCCmec IV(2B&c) [35], all isolates belonging to pulotype ST100 associated with a new variant of SCCmec IV (IVNv), which was positive by PCR for J1 specific region of the SCCmec subtype IVc and *ccdB* but negative for B mec complex [15], were further characterized for the mecA downstream vicinity with primers designed based on the sequence previously determined of strain ZH47-(ST100), accession AM292304, (File S1).

The primer sequences are as follows (location relative to the ZH47 sequence given in parentheses): i) *Tn*4001-1-F, 5’-GCC AAT CGC TTA ATT GGA GCC G-3’ (18.146 to 18.167) and *Tn*4001-1-R, 5’-ACT TCA TCT TCC CAA GGC GCT GT-3’ (18.854 to 18.832); amplifying the region IS1256l-aaC2(2)−apk6(2) within the *Tn*4001. ii) *Tn*4001-2-F, 5’-TGG CCA CGA CTT GTT CTT GG-3’ (21.164 to 21.183) and *Tn*4001-2-R, 5’-TCT GAT GTC TGC CCG AGG ACT-3’ (22.131 to 22.111); amplifying the region between the IS256R (*Tn*4001) and IS1272 from *mec* complex B and iii) *Tn*4001-3-F, 5’-ACC AAA CCC GAC AAC TAC AAC TAT-3’ (15.976 to 15.999) and *Tn*4001-3-R, 5’-GTG TCG TAA AGC TGG TGC CA-3’ (17.273 to 17.2554); amplifying the region between the *mecA* locus and IS256L, (*Tn*4001). The amplicons with the expected size were end sequenced and compared to the SCCmec IV(2B&c5) sequence, in the ZH47 strains-(ST100) [35]. All primers used for SCCmec IVNv characterization are shown in detail in (File S1).

**Statistical analysis**

Bacteriologic and patient data were compiled in an electronic database using Access (Microsoft). Data were analyzed with Epi Info version 6.0.4 software (Centers for Disease Control and Prevention, Atlanta, GA). Comparisons between groups were performed with chi-square test or Fisher’s exact test, as appropriate. *P*≤0.05 was considered statistically significant.

**Results**

**Temporal trends in CA-MRSA infections**

During 2007–2008, CA-MRSA accounted for 45% (99 CA-MRSA/221 CA-S. aureus) of community-associated *S. aureus* infections in pediatric patients cared for in three children's hospitals of Córdoba, [38% (36/94) in 2007 and 49% (63/127) in 2008]. Hence, these data indicate a significant increase of the annual prevalence of CA-MRSA infections since 2005 [33% (7/21) for a three-month period [12] and 25% (18/71) annually, *P*<0.01 for trend]. Additionally, the overall rate of CA-MRSA infections increased significantly (3.6 fold) accounting for 5.1 to 18.6 cases/100,000 annual visits in 2005 and 2008, respectively, *P*<0.0001 (Figure 1). In contrast, the incidence of CA-MSSA infections only increased 1.1 fold, from 16.2 cases in 2003 to 19.1 cases/100,000 annual visits in 2008. The number of children with invasive CA-MRSA infections in three Córdoba children’s hospitals increased significantly from 1.3 in 2005 to 11.1 cases/10,000 admissions in 2008, *P*<0.001.

Similar trends were observed yearly in one of these hospitals (CBAH1) upon analysis of the incidence (CA-MSSA, total CA-MRSA and invasive CA-MRSA infections, Figure 1) from 2003 to 2008. The characteristics, origin and incidence of CA-MRSA during 2007 in all hospitals are shown in Table 1. Importantly, the rates...
of CA-MRSA infections were highly variable among children from central, eastern and northern regions of Argentina, reaching an overall rate of 14.2 cases/100,000 annual visits (range 6.9–27) in 2007 (Table 1).

Community onset MRSA infections: Demographic and clinical data

The distribution of CA-MRSA infections (Córdoba, 2007–2008 and CSACHARG, 2007) and HACO-MRSA infections (CSACHARG, 2007) regarding patient’s clinical features, age and sex are shown in Table 2. Although children from Córdoba with CA-MRSA infections were significantly older than children from CSACHARG, both groups were comparable in sex, type and severity of the infections (Table 2).

Similar types of invasive CA-MRSA infections (n: 7) were detected in CBAH1 during 2003–2006: osteomyelitis (n: 2), arthritis (n: 1), pneumonia (n: 1), bacteraemia (n: 1), lymphadenitis (n: 1) and necrotizing fasciitis (n: 1).

Among selected children with HACO-MRSA infections (n: 51), multiple risk factors (≥2) were found in 35 (68%). The most common HRFs were: presence of indwelling catheters or percutaneous medical devices (85%) and hospitalization within the previous year (73%). In addition, 70% of the patients had chronic diseases, including immunodeficiencies (human immunodeficiency virus-HIV/acquired immunodeficiency syndrome-AIDS and malignancies), cardiovascular diseases, chronic pulmonary diseases, chronic renal insufficiency, diabetes, or chronic skin illnesses. These children were more likely to develop invasive infections (65% vs 33%) and presentation with sepsis (27% vs 13%) than children with CA-MRSA infections. Invasive HACO-MRSA cases included mainly lungs and bloodstream infections. In addition, they had significantly lower frequency of SSTI infections (12% vs 67%).

Moreover, 23% of HACO-MRSA isolates were obtained from surgical site infections (Table 2).

The isolates from invasive CA-MRSA infections from CBAH1-H3 and CSACHARG groups (total n: 104) were obtained from blood (74%), other normally sterile body sites including joints (8%), bone (8%), pleural fluid (6%), other body fluids (2%) and other sites (1% each).

Antimicrobial susceptibility profile of community-onset MRSA strains

From all CA-MRSA isolates (n: 312) recovered from the CBAH1-3 and CSACHARG groups during 2007–2008, 20% were resistant to at least one non-β-lactam antibiotic. Resistance to erythromycin (ERY-15%), clindamycin (CLI-14%, 93% of inducible resistance); gentamicin (GEN-8%), rifampin (RIF-2%) and chloramphenicol (CHL-1%) were detected. The HACO-MRSA isolates showed significantly (P<0.001) higher rates of resistance to ERY (45%), CLIN (42%, 23% inducible); GEN (43%) and RIF (14%) than the CA-MRSA isolates. In addition, resistance to ciprofloxacin (22%) and to trimethoprim-sulfamethoxazole (6%) were only detected among HACO-MRSA isolates. All isolates were susceptible to vancomycin by agar dilution method (MIC90, 1 µg/mL; MIC range, 0.25–2 µg/mL) and teicoplanin (MIC90, 2 µg/mL; MIC range, 0.25–4 µg/mL). From the total 363 CO-MRSA isolates evaluated, only 3 MRSA from children with HRFs (HACO-MRSA infections), were found to be h-VISA.
### Table 1. Characteristics, incidence of CA-MRSA infections and location of children’s hospitals of northern, eastern and central of Argentina, 2007.

<table>
<thead>
<tr>
<th>Children’s Hospitals</th>
<th>Province</th>
<th>Number of beds</th>
<th>Annual admission</th>
<th>Annual visitsb</th>
<th>Number of CA-MRSA&lt;sup&gt;c&lt;/sup&gt; 2007</th>
<th>Incidence&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>H1</td>
<td>Córdoba</td>
<td>230</td>
<td>10,375</td>
<td>119,365</td>
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<tr>
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<td>123,000</td>
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<td>8.1</td>
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<tr>
<td>H3</td>
<td>Córdoba</td>
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<td>1,775</td>
<td>107,000</td>
<td>10</td>
<td>9.3</td>
</tr>
<tr>
<td>H4</td>
<td>Santa Fe</td>
<td>96</td>
<td>8,000</td>
<td>220,000</td>
<td>29</td>
<td>13.2</td>
</tr>
<tr>
<td>H5</td>
<td>Buenos Aires</td>
<td>274</td>
<td>6,756</td>
<td>202,000</td>
<td>14</td>
<td>6.9</td>
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<tr>
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<td>Buenos Aires</td>
<td>142</td>
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<td>264,938</td>
<td>33</td>
<td>12.5</td>
</tr>
<tr>
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<td>175,683</td>
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<td>3,273</td>
<td>214,091</td>
<td>24</td>
<td>11.2</td>
</tr>
</tbody>
</table>

<sup>a</sup>Number of all CA-MRSA isolates detected in the three Córdoba children’s hospitals during 2007 and those recovered in each hospital from the surveillance study for community onset S. aureus infections in children from Argentina-CSACHARG [16].

<sup>b</sup>Annual visits: include outpatient facility and emergency service.

<sup>c</sup>Incidence: Number of cases/100,000 annual visits.

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### Molecular characterization of community-onset MRSA strains: CA-MRSA and HACO-MRSA

The molecular characteristics [sequence type (ST, as defined by MLST), clonal complex (CC), agr allotype, PFGE, SCCmec and spa types, presence of pvl genes and virulence genes profile, along with the drug resistance pattern of the most predominant pulsotypes are shown in Table 3. The distribution of the most predominant genotypes of CO-MRSA isolates involved in invasive and non invasive infections according to the epidemiologic case classification (CA-MRSA and HACO-MRSA) are shown in Table 4. The molecular characterization of the three h-VISA strains demonstrated that they belonged to three different MRSA clones (Table 3).

**CA-MRSA clone ST5-IV.** PFGE performed on 363 isolates (312 CA-MRSA and 51 HACO-MRSA) revealed a predominant major pulsotype, ("I": 79%, 285/363 isolates) with a more frequent subtype, I-1 (71%, 202/285). All isolates belonging to the pulsotype "I" were characterized as agr 2, ST5, except two isolates belonging to a new single locus variant (SLV) of ST5: ST1524, spa-t019 (91% of isolates) and related (t002, t045; t067; t1094). Most of them were associated with SCCmec type IV(2B), 96% with subtype IVa; 2% with subtype IVc and one isolate was nominated as SCCmec type IV non tyappable (IVNT), since it was positive by PCR for ccrB2 and mec gene complex class B, but negative for J1 regions: a, b, c, d, g and h (Table 3). Two isolates (118 subtype) were positive only for ccrC locus and negative for J1 region of SCCmec V and for other SCCmec regions analyzed [31] so they were tentatively designated as SCCmec related to Vr (Table 3). The pvl genes were not detected in 9% of the “I” pulsotype isolates (26/285) (Table 3 and Figure 2A). There was considerable diversity among the PFGE patterns of these isolates, demonstrating an ongoing evolution: 13 subtypes from 26 PVL<sup>+</sup> isolates vs 15 subtypes from 259 PVL<sup>−</sup> isolates. Unlike PVL<sup>+</sup> isolates, within the PVL group, spa-t002 (85%) was much more prevalent than spa-t311 (4%); and did not have the sea gene.

Additionally, all isolates belonging to pulsotype “I” recovered in CBAH1 during 2003–2006 (n: 20) were characterized as ST5, spa-t311, associated with SCCmecIVa as well as they harbour pvl and sea genes.

This clone represented 79% of all community-onset MRSA isolates (285/363) in both groups of infections, CA-MRSA (82%, 256/312) and HACO-MRSA (57%, 29/51) (Figure 2 and Table 4). Moreover, this pulsotype predominated among CA-MRSA isolated in CBAH1 during 2003–2006 (91%, 20/22). Longitudinal analysis of CA-MRSA infections at this hospital demonstrated that the significant increase of the incidence of both total CA-MRSA infections and invasive CA-MRSA infections were due to the dissemination of this single pulsotype “I” (ST5-SCCmecIV-PVL) increasing from 1 to 25 cases of CA-MRSA infections between 2003 and 2008 (Figure 1).

On the other hand, both groups, PVL<sup>+</sup> and PVL<sup>−</sup> variants, produced invasive infections in similar proportions (35%, 90 invasive infections/259 PVL<sup>−</sup> MRSA infections vs 35%, 9 invasive infections of 26 pulsotype “I”-PVL<sup>−</sup> MRSA infections; P = 0.96). Moreover, both groups were comparable regarding sepsis presentation (13%, 33/259 PVL<sup>+</sup> vs 12%, 3/26 PVL<sup>−</sup>; P = 0.8).

**Southwest Pacific (SWP) clone.** The second prevalent clone among total community-onset MRSA isolates (11%, 39/363) was the N pulsotype or Southwest Pacific (SWP) clone. It was characterized as agr 3, ST30 (4 isolates as ST196/SLV) belonging to CC30, spa-t019 and related t021, t6653 (new spa type described for the first time in this study) and spa-t975. Most isolates harbored SCCmecIVc, (97%) and all of them carried the pvl genes. This genetic background accounted for 11.5% (36/312) and 6% (3/51) of CA-MRSA and HACO-MRSA isolates respectively (Table 4). The molecular characteristics of this clone and its distribution in different regions of Argentina during 2007 are shown in Table 4 and Figure 2. In addition, this clone was significantly more frequent in Buenos Aires city and surrounding areas (21%) than in other regions of Argentina (average 8%, range ± 7–9, P = 0.009, Figure 2B).
Pediatric clone in Argentina: ST100-IVNv. A minor HA-MRSA clone, non-multiresistant to antibiotics, named pulsotype C or D by Sola et al and Corso et al, respectively, clonally related to the Pediatric clone by PFGE analysis [36,37] was also detected. It was characterized as agr 2, ST100, belonging to CC5, spa-t002, associated with a SCCmec IV new variant (SCCmec IVNv), and PVL+ [12,15].

In this study, Tn4001 was detected, integrated into the class B mec complex upstream of the mecA gene (class B mec gene complex) by means of PCR typing of different regions of SCCmecIV in representative isolates of the most frequent subtypes (File S1). Hence, this new variant of SCCmecIV, in addition to class B2 mec gene complex, also carries type 2 ec gene complex and a J1 region with homology to SCCmecIV, but unlike the SCCmecIV(2B&5), it lacks of an SCC with ccrC and contains dse gene.

This clone was significantly more frequent among HACO-MRSA infections (10%, 5/51) than among total CA-MRSA infections (2%, 5/312), P = 0.009, (Table 4).

South American USA300 clone. Four CA-MRSA isolates (one detected in Buenos Aires in 2007 and three in Córdoba during 2007–2008) were clonally related by PFGE to the USA300 MRSA epidemic strain. They belonged to ST8, CC8, spa-t008 and to agr 1. Three of these isolates were associated with SCCmecIVc and one, with subtype IVa. All of them carried the pvl, hsdS and ser genes. In contrast, all lacked of the mecA gene, which is an indicator of the presence of the ACME. The isolate associated with SCCmecIVa also carried sed and seh genes (Figure 2A and Table 3).

Other minor clones. Among CA-MRSA isolates, other minor pulsotypes were also detected accounting cumulatively for only 4% of isolates (n: 10, less than 2% each), (Table 3). Among these, two isolates were characterized as ST188/CC1 and associated with SCCmecV constituting the first time that this genotype is reported in America [19].

The MRSA strains traditionally associated with nosocomial infections in Argentina [12,15,36,37] only accounted for 37% of the total HACO-MRSA infections (Cordobes/Chilean-pulsotype
## Table 3. Characteristics of MRSA clones isolated from children with community onset MRSA infections (CO-MRSA), Argentina.

<table>
<thead>
<tr>
<th>Clone</th>
<th>Related to Pediatric clone</th>
<th>Southwestern Pacific (SWP) clone</th>
<th>South American USA300 clone</th>
<th>Cordobes/Chilean clone</th>
<th>Brazilian clone</th>
</tr>
</thead>
<tbody>
<tr>
<td>n: 285</td>
<td>n: 285</td>
<td>ST100: 10 (100)</td>
<td>ST3b: 35 (90); ST196: 4 (10)</td>
<td>ST5: 12 (100)</td>
<td>ST239: 3 (100)</td>
</tr>
<tr>
<td>Molecular characteristics</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>agr allelic group</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>ST n (%)a</td>
<td>ST5: 283 (99); ST1S242 (1)</td>
<td>ST100: 10 (100)</td>
<td>ST3b: 35 (90); ST196: 4 (10)</td>
<td>ST5: 12 (100)</td>
<td>ST239: 3 (100)</td>
</tr>
<tr>
<td>Clonal complex</td>
<td>5</td>
<td>10</td>
<td>39</td>
<td>4</td>
<td>12</td>
</tr>
<tr>
<td>SCCmec type n (%)a</td>
<td>I-V: 275 (96), IVc: 6 (2), IVNT: 1; W: 2; N: 1</td>
<td>I-V: 28: IVC: 38 (97), IVc: 1 (3)</td>
<td>IV(2B): IVc: 3 (75), IVa: 1 (25)</td>
<td>I</td>
<td>IIA</td>
</tr>
<tr>
<td>PFGE type</td>
<td>C</td>
<td>N</td>
<td>USA300</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pvl n (%)b</td>
<td>PVL: 259 (91)</td>
<td>PVL: 26 (9)</td>
<td>PVL: 39 (100)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PFGE Subtype: n (%)a</td>
<td>I-1: 202 (71) and 14 minor subtypes</td>
<td>I-2: 7 (2.5) and 12 minor subtypes</td>
<td>C-1: 2 (20) and 7 minor subtypes</td>
<td>USA300, 4 subtypes</td>
<td>B15: 2 (66) and 1 subtype</td>
</tr>
<tr>
<td>RIDOM spa type: n (%)a</td>
<td>t131: 259 (100)</td>
<td>t002: 22 (85); t131, t045, t094</td>
<td>t102: 10 (100)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Virulence genes profile</td>
<td>seas-egc-lukADE</td>
<td>egc-lukADE</td>
<td>egc-lukADE</td>
<td>lukDE-sek-bsa: 3 (75); lukDE-sek-sej-sej: 1 (25)</td>
<td>lukDE-bsa</td>
</tr>
<tr>
<td>Drug resistance</td>
<td>ERY (14)</td>
<td>GEN (100)</td>
<td>GEN (5)</td>
<td>GEN (92)</td>
<td>GEN (100)</td>
</tr>
<tr>
<td>non-β-Lactam (%)a</td>
<td>CLIN (14)</td>
<td>ERY (50)</td>
<td>ERY (18)</td>
<td>CIP (75)</td>
<td>CIP (100)</td>
</tr>
<tr>
<td></td>
<td>GEN (8)</td>
<td>CLIN (10); CLINc (30)</td>
<td>CLIN (18)</td>
<td>ERY (100)</td>
<td>ERY (100)</td>
</tr>
<tr>
<td></td>
<td>RIF (1)</td>
<td>RIF (30)</td>
<td>CHL (3)</td>
<td>CLIC (92)</td>
<td>CLIC (100)</td>
</tr>
<tr>
<td></td>
<td>h-VISA (1)</td>
<td>h-VISA (1)</td>
<td>h-VISA (1)</td>
<td>RIF (17)</td>
<td>RIF (66)</td>
</tr>
<tr>
<td>MINOR CLONES, n: 10</td>
<td>ST188/CC1, n: 2</td>
<td>PFGE type T1, SCCmecVr, spa-t1189, agr 1, cna&quot;, PVL &quot;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ST197/CC8, n: 3</td>
<td>PFGE type R1, SCCmecVNT, spa-t1148, agr 1, PVL &quot;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ST207/CC509, n: 3</td>
<td>PFGE type Y1, SCCmecVs, spa-t525, agr 3, PVL &quot;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ST198/Singleton, n: 1</td>
<td>PFGE type S1, SCCmecVs, spa-t1194, agr 3, PVL &quot;</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*agr type, type of accessory gene regulator, ST: Sequence Type, SCCmec: Staphylococcal Cassette Chromosome mec, PFGE, Pulsed Field Gel Electrophoresis; RIDOM spa type: staphylococcal protein A (spa) type assigned through the RIDOM database (http://spaserver.ridom.de).  
a(%) total number and % of strains with this molecular characteristic [PFGE subtype (only those more frequent are indicated) or ST or spa type or SCCmec type]. % is not expressed when only one isolate with this characteristic was detected.  
bPVL: Panton-Valentine leukocidin genes (lukS-PV-lukF-PV): indicated as number and % of isolates harboring (PVL) or not (PVL -) pvl genes.  
cVirulence genes profile: From all virulence genes analyzed, only those detected are indicated (number and % of positive isolates is expressed when not all isolates harbor this virulence factor).  
dDrug resistance non-β-Lactam: Gentamicin (GEN), Ciprofloxacin (CIP), Erythromycin (ERY), Clindamycin (CLIN) and CLI: constitutive and inducible resistance to macrolides, lincosamide and streptogramine B, respectively. RIF: rifampin (RIF), chloramphenicol (CHL), trimethoprim/sulfamethoxazole (SXT) and minocycline (MIN) (%) of strains resistant to these antibiotics within each pulsotype is indicated when more than one isolate was detected. h-VISA (1): means one isolate belonging to this clone with phenotype h-VISA.  
eIV NT: SCCmec type IV non tolerant.
A-ST5-I-t149, 21%; Pediatric-pulsotype C-ST100-IVNv-t002: 10% and Brazilian-pulsotype B-ST239-IIIA-t037: 6%, Figure 2A

**Discussion**

Laboratory-based surveillance of community-associated *S. aureus* infections in three children's hospitals in Córdoba-Argentina during 2007–2008 revealed a significant increase of the annual prevalence of CA-MRSA infections since 2005 (49%, 2008 vs 25%, 2005). Moreover, the incidence of CA-MRSA infections among children tripled over that time frame, associated with an 8.5-fold increase in invasive CA-MRSA infections, despite the almost stable overall rate of CA-MSSA infections (1.1 fold increase). A similar pattern with a steady annual increase of CA-MRSA infections and a slight increase of CA-MSSA infections was observed. A similar pattern with a steady annual increase of CA-MRSA infections over time (2003–2008) in Córdoba, Argentina, as reported in some regions of United States, particularly in patients with skin and soft-tissue infections, CA-MRSA strains are adding to the burden of CO- *S. aureus* infections, instead of replacing other *S. aureus* strains [8,38].

Unlike in North America, few studies have documented the epidemiology of MRSA infections in the community among Latin-American children [1,12,39]. This study provides the first comprehensive description carried out in Argentina about the evolution of CA-MRSA infections over time (2003–2008) in children from Córdoba.

The types of CA-MRSA infections detected in this study are similar to those described worldwide. Nevertheless, it is important to remark the high proportion of children with invasive CA-MRSA infections in Argentina (33%) compared to previous reports from other countries (1–12%) [20,40]. Regarding HACO-MRSA infections, most children had some type of chronic disease and multiple HRFs, were more susceptible to surgical site and invasive infections as well as sepsis, and had significantly lower frequency of SSTIs compared to children with CA-MRSA infections, in agreement with other studies [4,21,41].

Although variable rates of CA-MRSA infection among children from different regions of Argentina were detected in 2007, one of the most striking finding of our study is that one major CA-MRSA clone (I-ST3-SSCmecPVL+PVL+) is currently predominating in both groups: community-associated cases (82%) and healthcare-associated, community-onset cases (57%) in hospitals of northern, eastern and central regions of our country, contrasting with other countries [4].

Therefore, the wide dissemination of this clone over all these regions of Argentina, along with the continued steep rise of its incidence over time in Córdoba, confirm its epidemic nature. Importantly, these results suggest that many children, despite documented contacts with healthcare delivery, are frequently infected with a CA-MRSA clone. This finding, though indirectly, suggests that this clone has entered into the healthcare settings and thus, represents a potentially serious public health challenge for next years, as described elsewhere for other CA-MRSA clones [8–10].

The molecular characteristics shared by the isolates belonging to this major clone corresponded largely to those reported in our previous paper [12], namely, *spa* ST5, *spa*-t311 and related ones, *SCCmec* IV (96% IVa). In addition, most of these isolates carried *pel* and *sea* genes (91%). Although most of CA-MRSA infections (invasive and non invasive) caused by the “I” clone corresponded largely to those reported in our previous paper [12], specifically, to this major clone corresponded largely to those reported in our previous paper [12].

Recently, Nuebl et al [42], by using single-nucleotide polymorphism analysis, demonstrated that much of the population structure of ST5 is local, with multiple independent introductions of *SCCmec*, producing a regional-specific evolution of particular sublineages. In agreement with that hypothesis, in previous studies we demonstrated the presence of ST5-PVL+ isolates, both groups, PVL+ and PVL−, were comparable regarding the proportions of invasive infections and presentation with sepsis. Hence, these results lead to hypothesize that the presence of *pel* and *sea* genes associated with *spa*-t311 in this lineage would be more related to a possible increase in transmissibility and fitness of this CA-MRSA clone, rather than to its virulence. Further studies are needed to confirm this hypothesis.

Table 4. Genotypes of methicillin resistant *S. aureus* (MRSA) isolates recovered from children with Community-onset infections (invasive and non-invasive) in central, northern and eastern regions of Argentina, by epidemiologic case classification.

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Total CO</th>
<th>Community-Associated (CA)</th>
<th>Healthcare-associated CO (HACO)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I-ST5-IV</td>
<td>285 (79); 99 (72)</td>
<td>57 (90); 14 (82)</td>
<td>170 (80); 61 (79)</td>
</tr>
<tr>
<td>N-ST30-IV</td>
<td>39 (11), 21 (8)</td>
<td>3 (5), 2 (12)</td>
<td>3 (8), 1 (10)</td>
</tr>
<tr>
<td>USA300-ST8-IV</td>
<td>4 (1); 3 (2)</td>
<td>2 (3); 1 (6)</td>
<td>1 (3); 1 (10)</td>
</tr>
<tr>
<td>C-ST100-IVNv</td>
<td>10 (3); 6 (4)</td>
<td>—</td>
<td>4 (2); 2 (2.5)</td>
</tr>
<tr>
<td>A-ST5-I</td>
<td>12 (3); 10 (7)</td>
<td>—</td>
<td>1 (0.5); 1 (1)</td>
</tr>
<tr>
<td>B-ST239-IIIA</td>
<td>3 (1); 2 (1.5)</td>
<td>—</td>
<td>3 (6); 2 (6)</td>
</tr>
<tr>
<td>OTHERS</td>
<td>10 (2)</td>
<td>1 (2)</td>
<td>1 (3)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>363</td>
<td>63</td>
<td>312</td>
</tr>
</tbody>
</table>

The most striking finding of our study is that one major CA-MRSA clone (I-ST3-SSCmecIV-PVL+) is currently predominating in both groups: community-associated cases (82%) and healthcare-associated, community-onset cases (57%) in hospitals of northern, eastern and central regions of our country, contrasting with other countries [4]. Therefore, the wide dissemination of this clone over all these regions of Argentina, along with the continued steep rise of its incidence over time in Córdoba, confirm its epidemic nature. Importantly, these results suggest that many children, despite documented contacts with healthcare delivery, are frequently infected with a CA-MRSA clone. This finding, though indirectly, suggests that this clone has entered into the healthcare settings and thus, represents a potentially serious public health challenge for next years, as described elsewhere for other CA-MRSA clones [8–10].

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Figure 2. Molecular characteristics and proportion from different regions of Argentina of dominant community methicillin-resistant *Staphylococcus aureus* clones. A. PFGE pattern analysis for representative isolates belonging to the most prevalent subtypes of community-onset-MRSA clones (CA-MRSA and HA-MRSA) detected in central, eastern and northern regions of Argentina during 2007–2008. The schematic presentation of *Smal* restriction patterns (middle) and dendrogram (left) by the unweighted-pair group method using average linkage clusterings are shown. Genotypes are denoted as subtype (by PFGE)-ST (by MLST)-SCC* mec* type-*spa* type (right). CA-MRSA clones appear in gray. The presence (+) or absence (−) of *pvl* genes (by PCR) is also indicated for each subtype; strains with and other without *pvl* genes belonging to the same PFGE subtype (I9) are indicated as +/−. The PFGE pattern of USA300-0114 (ST8-IVa-t008-ACME+) is shown for comparison purposes. The first (A1-ST5-I-t149-Cordobes/Chilean), second (B1-ST239-IIIA-t037-Brazilian) and third (C1-ST100-IVNv-t002-Pediatric) more frequent HA-MRSA clones in our country, detected among community-onset MRSA infections, are also shown (dotted gray). B: Proportion of CA-MRSA clones among representative isolates from different regions of Argentina in 2007.
doi:10.1371/journal.pone.0030487.g002
This clone was significantly more frequent in Buenos Aires city than one among CA-MRSA isolates in 2004–2006 [13]. In our study, this clone (ST30-IV) had already been detected as a minor in Buenos Aires, where it is highly prevalent at community and hospital settings [46]. In 2004, it was more commonly isolated from HACO-MRSA cases than from CA-MRSA infections (10% vs 2%), as shown herein. The ST100 genotype was also identified in a MRSA isolate from Zurich in 2003 (MRSA-ZH47), associated with the new SCCmecIV-2B&5 subtype [35]. In this study, we demonstrated for the first time that the SCCmec associated with pulsotype C (Pediatric clone)-ST100 in Argentina is closely related to SCCmecIV-2B&5. Therefore, to classify this new variant of SCCmecIV, it would be necessary to determine the complete nucleotide sequence, which is currently in progress.

The pandemic Southwest Pacific-(SWP) clone or USA1100 was one of the minor clones found, accounting for 11.5% of CA-MRSA isolates. It was characterized as pulsotype N, ST30, spa-t019 and related ones. Most isolates harbored SCCmecIVc (97%) all of them carried pol genes and adhesins ssa and bfp genes. Since mid-1990s, this CA-MRSA clone, ST30-IV-PVL+ with different pulsotypes or spa types or SCCmecIV subtypes has been reported from many regions of the world [1,46]. In Buenos Aires, this clone (ST30-IV) had already been detected as a minor one among CA-MRSA isolates in 2004–2006 [13]. In our study, this clone was significantly more frequent in Buenos Aires city and surrounding areas (21%) than in other regions analyzed, where it accounted for 8% of CA-MRSA isolates in 2007. Although its frequency is still very low, this situation might suggest that this CA-MRSA clone is spreading from Uruguay, where it is highly prevalent at community and hospital settings [11,51].

Recently, the South American USA300 MRSA-(ST3-SCCmecIV-t008-PVL+)-ACME− clone was described as the predominant (96%) CA-MRSA in northern areas of South America between 2006 and 2008 [14]. An additional important issue of this study was the finding of the first four cases of CA-MRSA infections caused by this highly virulent CA-MRSA clone in the southern cone of Latin America. ACME-negative variants of USA300 also appear to be common in Western Australia and in Spain [52–54], though we were unable to find eventual epidemiological association between Argentinean strains and those from these countries.

Our results confirmed the high prevalence of MRSA infections in the community in Argentinean children, so the characteristics of antimicrobial susceptibility of these strains must be considered for the selection of empiric therapies. Although most CA-MRSA isolates were susceptible to all non-β-lactam antibiotics, inducible resistance to clindamycin (14%), resistance to gentamicin (8%), rifampin (1%) and chloramphenicol (1%) were detected in isolates belonging to ST5-IV-PVL+ CA-MRSA clone (Table 3). It is important to remark that this clone’s resistance to antibiotics was only represented by clindamycin, just in 10% of isolates, as described in the previous surveillance performed in Córdoba in 2005 [12]. Hence, these results support the ability of the ST5-IV-PVL+ CA-MRSA clone to acquire new resistance determinants.

Importantly, in our study, three strains with h-VISA phenotype from children with HRFs were detected. One of these cases and another one detected in an adult patient without HRFs in 2009 were PVL− variants of ST3-SCCmecIV-IV CA-MRSA clone. Considering the high proportion of children with invasive CA-MRSA infections caused by this clone, along with the fact that vancomycin is still the cornerstone used for the treatment of this type of CA-MRSA infections [7], this finding constitutes another concern for public health.

Our study was limited by the use of the CDC criteria to identify patients with infections caused by CA-MRSA isolates, which appear to underestimate the burden of diseases produced by these strains. Additionally, evidence from this study, though indirect, indicate that CA-MRSA strains have entered the hospital settings of Argentina and probably the burden of CA-MRSA is even greater than shown here.

In conclusion, we observed a significant increase in the incidence of invasive and non-invasive CA-MRSA infections, in children of Córdoba between 2005 and 2008. This raise could be attributed entirely to the rapid dissemination of an epidemic CA-MRSA clone characterized as I-STS-SCCmecIVa-spa-t311-PVL+. Importantly, this highly virulent clone with capacity to express the phenotype h-VISA, predominates in central, northern and eastern regions of Argentina and still more worryingly, it has probably entered the hospital settings. The specific virulence, antibiotic resistance and transmission traits of a CA-MRSA clone distinct from USA300 would need to be identified in order to recognize priority targets for drug and vaccine development. These virulent clones represent a new threat to public health in Argentina and probably worldwide, warranting close monitoring during next years.

Supporting Information

File S1 Molecular characterization of SCCmec associated with PFGE pattern C and sequence type ST100: Pediatric clone in Argentina. (PDF)

Acknowledgments

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Author Contributions

Conceived and designed the experiments: CS HP JLB. Performed the experiments: CS HP ALE AJM AG IK CC HI JLB. Analyzed the data: CS HP JLB. Contributed reagents/materials/analysis tools: CS HP IK AV JLB. Wrote the paper: CS JLB. Patients’ clinical care, collection of clinical and epidemiology data, microbiology diagnostic: Study Group of CA-MRSA in Children, Argentina-2007.

References