Community-associated methicillin-resistant Staphylococcus aureus in children treated in Uruguay

Lorena Pardo1†, Magdalena Vola1†, Marina Macedo-Viñas1†, Virginia Machado1, Dianna Cuello1, Marta Mollerach2, Marta Castro1, Catalina Pírez3, Gustavo Varea1††, Gabriela Algora1,4†

1Departamento de Bacteriología y Virología, Instituto de Higiene, Facultad de Medicina, Universidad de la República, Montevideo, Uruguay
2Cátedra de Microbiología, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina
3Departamento de Pediatría, Facultad de Medicina, Universidad de la República, Centro Hospitalario “Pereira Rossell”, Montevideo, Uruguay
4Laboratorio de Bacteriología, Centro Hospitalario “Pereira Rossell”, Montevideo, Uruguay

* L.P.; M.V. and M.M. contributed equally to the work.
† G.V. and G.A. contributed equally to the production of this paper.

Abstract
Introduction: Staphylococcus aureus produces a variety of diseases among children, ranging from skin and soft tissue infections to invasive life-threatening diseases. Since 1990, an increasing number of diseases produced by community-associated methicillin-resistant S. aureus (CA-MRSA) isolates have been reported. The aim of this study was to describe the importance and the microbiological characteristics of S. aureus isolates recovered from children treated at the Hospital Pediátrico del Centro Hospitalario “Pereira Rossell” (HP-CHPR); focusing on invasive diseases caused by CA-MRSA isolates, as well as some clinical aspects of the diseases they have produced.

Methodology: One hundred and twenty-five S. aureus isolates recovered from the HP-CHPR between 2003 and 2006 from children with invasive (n=89) and superficial diseases (n=36) were included. Genotypic and phenotypic characteristics of S. aureus isolates and relevant clinical aspects of each child were studied.

Results: CA-MRSA isolates accounted for 73% of all S. aureus recovered from invasive (mainly bone and joint) infections, pneumonia and bacteraemia. The most common CA-MRSA strain recovered from invasive (n=65) and superficial (n=36) diseases had the following features: pulotype A (type USA1100), SCCmec cassette type IV, Panton-Valentine Leukocidin genes positive, susceptibility to trimethoprim-sulfamethoxazole without the inducible macrolide-lincosamide-streptogramin B (iMLSb) resistance phenotype. No association between genotypic characteristics of invasive CA-MRSA isolates and clinical outcomes was found.

Conclusions: CA-MRSA isolates produced a wide spectrum of invasive diseases in a public paediatric hospital between 2003 and 2006. Microbiologic characterization suggests the spread of an adapted CA-MRSA clone lacking erm genes.

Key words: Staphylococcus aureus; methicillin resistance; pediatric diseases; Uruguay


(Received 18 August 2011 – Accepted 05 April 2012)

Copyright © 2013 Pardo et al. This is an open-access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Introduction
Staphylococcus aureus produces a variety of diseases among children, ranging from skin and soft tissue infections (SSTIs) to invasive life-threatening diseases [1-3]. An important characteristic of S. aureus is its capacity to acquire resistance to antibiotics, making it difficult to choose a suitable empiric antimicrobial therapy. Since 1990, an increasing number of diseases produced by methicillin-resistant S. aureus (MRSA) have been reported, affecting individuals without the classic risk factors linked to the acquisition of health-care associated methicillin-resistant S. aureus (HA-MRSA) strains [1,4-6]. These strains are now known as community-associated methicillin-resistant S. aureus (CA-MRSA) in contrast to HA-MRSA. CA-MRSA isolates are often only resistant to methicillin; they harbor SCCmec types IV, V or VII and most of them carry lukS-F genes [4].

In recent years, there has been a marked increase in the rate of CA-MRSA infections among children and adults [7,8] in Uruguay. According to data from the Pediátrico del Centro Hospitalario “Pereira
Rossell” (HP-CHPR) hospital 2% of S. aureus isolates recovered in 2001 were MRSA; in 2003 they accounted for 35%, and the figure rose to more than 50% in 2004.

The aim of this study was to describe the importance and the microbiological characteristics of S. aureus isolates recovered from children treated at the HP-CHPR; focusing on invasive diseases caused by CA-MRSA isolates, as well as some clinical aspects of the diseases they produce.

Methodology
Setting
The HP-CHPR is a 300-bed tertiary reference centre for all medical and surgical pediatric specialties. It provides high-level annual care to more than 300,000 children under 15 years old. Children receiving medical care at the HP-CHPR often come from low-income households.

Children and bacterial isolates
We analyzed 125 S. aureus isolates recovered between January 2003 and October 2006 from 125 children who sought medical attention at the HP-CHPR. Isolates obtained from children younger than 28 days old and those from children over the age of 15 years were excluded from the study. All S. aureus isolates (n = 89) recovered from invasive infections (e.g., bone, blood, cerebrospinal, pleural, and synovial samples) and 36 randomly selected MRSA isolates recovered in the same period from children with superficial infections, were also included in this study. Clinical information of each child such as age, gender, site of infection, admission to intensive care unit (ICU), and outcome were obtained from medical records.

Isolate identification and antimicrobial susceptibility testing
S. aureus isolates were identified by standard phenotypic procedures (colony morphology, Gram stain, catalase test, DNase activity, presence of clumping factor, and protein A by the latex agglutination test). MRSA strains were defined as CA-MRSA when they met the following criteria: they harboured SCCmec type IV, V or VI and were either susceptible to all non-beta-lactam antibiotics, showed resistance only to erythromycin, or displayed the inducible macrolide-lincosamide-streptogramin B (iMLS\textsubscript{B}) phenotype; they were isolated within 48 hours of admission to the HP-CHPR; and they were isolated from a child who had no previous history of hospitalization, surgery, dialysis or residence in a long-term care facility within one year of the MRSA culture date, or who had no permanent in-dwelling catheter or percutaneous medical device and no known prior positive culture for MRSA. Isolates were defined as HA-MRSA when they carried SCCmec type I, II or III, displayed a multiple antibiotic resistance profile, and they were isolated from a child who presented with any of the risk factors described above [4].

Antimicrobial susceptibility was determined using the disk-diffusion method, following recommendations from the Clinical and Laboratory Standards Institute [9]. The antibiotics tested were oxacillin (OXA), gentamicin (GM), trimethoprim/sulfamethoxazole (TMP-SMX), erythromycin (ERY), and clindamycin (CLI) (Oxoid Ltd., Basingstoke, Hampshire, UK). S. aureus strain ATCC 25923 was used as quality control. A double disk diffusion test was performed to determine the iMLS\textsubscript{B} phenotype [9]. The presence of PBPs in MRSA isolates was determined by commercial latex agglutination test (Oxoid).

S. aureus isolates were stored in skimmed milk frozen at -20°C and sent to the Department of Bacteriology and Virology of the School of Medicine, Universidad de la República, for genotyping.

Genotyping
Bacterial DNA was obtained from isolated colonies using the Wizard genomic DNA preparation kit (Promega, Madison, WI, USA) using 20 mg/mL lysostaphin (Sigma-Aldrich, St. Louis, MO, USA) in the cell-lysis step [10]. The presence of collagen-binding adhesin (cna) and Panton-Valentine Leukocidin (PVL) lukS-F genes was determined by PCR according to previously described procedures [11,12]. The SCCmec cassette type and presence of the mecA gene were determined by a multiplex PCR [13, 14].

DNA macrorestriction and separation of fragments by pulsed field gel electrophoresis (PFGE) were completed using a standardized protocol for the 125 S. aureus isolates [15]. DNA was digested with SmaI (New England Biolabs, Beverly, MA, USA) and PFGE conditions were 6 V/cm at 11.3°C for 23 hours with pulses ranging from 5 to 35 seconds. Electrophoresis was performed using a CHEF DR II instrument (Bio-Rad, Hercules, CA, USA). Band patterns were visually interpreted according to previously published criteria [16].
Table 1. Invasive S. aureus isolates recovered from children treated at the HP-CHPR between 2003 and 2006

<table>
<thead>
<tr>
<th>Infection site</th>
<th>MRSA No. of CA-MRSA Isolates</th>
<th>MRSA No. of HA-MRSA Isolates</th>
<th>MSSA No. of MSSA Isolates</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone and Joint infections</td>
<td>29</td>
<td>2</td>
<td>13</td>
<td>44</td>
</tr>
<tr>
<td>Bacteraemia</td>
<td>14</td>
<td>0</td>
<td>7</td>
<td>21</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>19</td>
<td>0</td>
<td>1</td>
<td>20</td>
</tr>
<tr>
<td>CNS</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Catheter infection</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Endocarditis</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>TOTAL</td>
<td>65</td>
<td>3</td>
<td>21</td>
<td>89</td>
</tr>
</tbody>
</table>

* The criteria used to distinguish between CA-MRSA and HA-MRSA isolates are included in the text [4].

Multilocus sequence typing (MLST)
Multilocus sequence typing (MLST) was performed on two randomly selected CA-MRSA strains belonging to pulsotype A, as previously published by Enright et al. [17]. PCR products were sequenced at the Instituto Pasteur de Montevideo (Montevideo, Uruguay) and the obtained sequences were analyzed using MLST website (http://www.mlst.net).

Statistical analysis
Fischer’s exact test was used to investigate a possible association between the presence of virulence factors and outcome of invasive diseases. A P value < 0.05 was considered statistically significant.

Results
Microbiological characteristics of S. aureus isolates recovered from invasive infections
Out of 89 S. aureus isolates recovered from children with invasive infections, 21 (23.6%) were susceptible to oxacillin (MSSA) while 68 (76.4%) were resistant (MRSA) (Table 1). All MRSA isolates expressed a PBP2a protein and carried the mecA gene.

Three of the sixty-eight (4.4%) MRSA isolates were defined as HA-MRSA. One HA-MRSA isolate was susceptible to TMP-SMX, carried SCCmec type I, and visually its PFGE profile closely resembled the previously described MRSA “Cordobes clone” (Figure 1, lane 3) [18]. The other two isolates were resistant to TMP-SMX; both carried SCCmec type III and showed a different PFGE profile (Figure 1, lanes 1 and 2). The remaining 65 MRSA isolates were defined as CA-MRSA (Table 1) and 29 (44.6%) showed an iMLSb phenotype. All 65 CA-MRSA isolates carried SCCmec type IV; 52 isolates displayed PFGE pulsotype A (PFGE profile type USA1100) (Figure 1, lane 8); and isolates 55 and 57 contained lukS-F and cna genes, respectively (Table 2). Both CA-MRSA isolates analyzed by MLST belonged to ST30.

Four out of 21 MSSA isolates recovered from invasive infections were resistant to ERY, GM or CLI but none showed the iMLSt phenotype. Six isolates were positive for the lukS-F genes (Table 2). Pulsotype D (Figure 1, lanes 6 and 7) was most frequently found in MSSA isolates (n = 9; there were 4 pulsotype A; and 2 pulsotype C isolates as well). All remaining MSSA isolates showed different PFGE profiles that did not match any previously observed profile in this study.

Figure 1. Representative PFGE profiles of Staphylococcus aureus isolates recovered from children treated at the HP-CHPR between 2003 and 2006
Clinical aspects of infection and their association with S. aureus microbiological characteristics

The median age of children included in the study was 63 months (5.25 years old); 59% were male. Bone and joint infections accounted for 50% of invasive disease (n = 44), followed by bacteraemia 23.5% (n = 21) and pneumonia 22.4% (n = 20). CA-MRSA isolates were recovered from bone and joint infections (n=29, 66%); bacteraemia (n = 14, 67%); pneumonia (n = 19, 95%); central nervous system infections (n = 2) and endocarditis (n = 1) (Table 1). Twenty-three of the 65 children with CA-MRSA invasive infection were admitted to the ICU. Nineteen of the CA-MRSA isolates recovered from these children belonged to pulsortype A (USA1100) and 19 isolates also carried lukS-F and cna genes (Table 3).

Over one-third (8 of 23; 34.7%) of the children admitted to the ICU died. Clinical diagnosis of the deceased children was sepsis (n = 3), pneumonia with empyema (n = 3), and bone and joint infection (n = 2). Isolates 6 and 7 recovered from these children carried the lukS-F and cna genes, respectively. No deaths were reported in the remaining 42 children with CA-MRSA invasive infections not admitted to the ICU (Table 3). No children with invasive infections caused by MSSA isolates (n = 21) or HA-MRSA isolates (n = 3) were admitted to ICU and no deaths were reported in this group (Table 1).

No children with superficial infection (n = 36) were admitted to the ICU and no associated deaths were registered in this group. CA-MRSA isolates were recovered from abscesses (n = 20), cellulitis (n = 6), and other superficial skin infections (n = 10). Thirty-two of the 36 CA-MRSA isolates recovered from superficial infections were admitted to ICU.

Table 2. Microbiological characteristics of 125 S. aureus isolates recovered from children with invasive and superficial infections treated at the HP-CHPR between 2003 and 2006

<table>
<thead>
<tr>
<th>Genotypic or phenotypic characteristic</th>
<th>CA-MRSA Invasive (n=65)</th>
<th>Superficial (n=36)</th>
<th>HA-MRSA Invasive (n=3)</th>
<th>MSSA Invasive (n=21)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nº. (% of isolates with positive result to:</td>
<td>Nº. (% of isolates with positive result to:</td>
<td>Nº. of isolates with positive result to:</td>
<td>Nº. (% of isolates with positive result to:</td>
</tr>
<tr>
<td>LukS-F genes</td>
<td>57 (87.6)</td>
<td>30 (83.3)</td>
<td>0</td>
<td>6 (28.5)</td>
</tr>
<tr>
<td>cna gene</td>
<td>55 (84.6)</td>
<td>29 (80.5)</td>
<td>1</td>
<td>3 (14.2)</td>
</tr>
<tr>
<td>SCCmec cassette type IV</td>
<td>65 (100)</td>
<td>36 (100)</td>
<td>0 ^</td>
<td>NA b</td>
</tr>
<tr>
<td>Pulsotype A (USA1100)</td>
<td>52 (80)</td>
<td>29 (80.5)</td>
<td>0</td>
<td>4 (19)</td>
</tr>
<tr>
<td>iMLSB phenotype</td>
<td>29 (44.6)</td>
<td>11 (30.5)</td>
<td>0</td>
<td>0 (0)</td>
</tr>
<tr>
<td>TMP-SMX susceptibility</td>
<td>65 (100)</td>
<td>36 (100)</td>
<td>1</td>
<td>21 (100)</td>
</tr>
</tbody>
</table>

^One isolate carried SCCmec cassette type I and the other two isolates carried SCCmec cassette type III; ^b Not applicable

Table 3. Outcome of the children with CA-MRSA invasive infections treated at the HP-CHPR between 2003 and 2006 and microbiological characteristics of their isolates

<table>
<thead>
<tr>
<th>Children with CA-MRSA invasive infections (n = 65)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Admitted to ICU (n = 23)</td>
</tr>
<tr>
<td>Nº (%) of isolates with lukS-F genes</td>
</tr>
<tr>
<td>Nº (%) of isolates with cna gene</td>
</tr>
<tr>
<td>Nº (%) of isolates with pulsotipo A (USA1100)</td>
</tr>
</tbody>
</table>

| Deaths                                            | 8 (34.7) | 0 (0) |

Microbiological characteristics of MRSA isolates recovered from superficial infections

All MRSA isolates (n = 36) recovered from superficial infections were identified as CA-MRSA and carried the type IV SCCmec cassette. Of the 36 isolates, 29 and 30 carried the lukS-F and cna genes, respectively (Table 2). Twenty-nine strains corresponded to pulsortype A (PFGE profile type USA1100) (Figure 1, lane 5).

Overall, 40 out of 101 CA-MRSA isolates (invasive n = 29 and superficial n = 11) displayed the iMLSB phenotype (Table 2). Figure 2 shows the distribution of these 40 isolates over the years of isolation.
out of 36 isolates carried lukS-F genes; 29 isolates carried the cna gene; and 29 isolates belonged pulsotype A (USA1100) (Table 2).

Using the Fisher’s exact test, no significant association was found between clinical outcome (death or recovery and invasive disease; n = 89) and the presence or absence of lukS-F and cna genes ($P = 0.428$ and $P = 0.712$, respectively).

**Discussion**

We analyzed all the invasive *S. aureus* infections diagnosed at the HP-CHPR over a three-year period. CA-MRSA isolates accounted for 73% (65/89) of these infections and the case fatality rate (34.7%) was similar to that previously reported [19-21]. CA-MRSA isolates produced a wide spectrum of invasive diseases; however, MSSA isolates were mainly responsible for bacteraemia and osteoarticular infections. The proportion of bone and joint infections and of bacteraemias caused by CA-MRSA was similar (66% and 67%, respectively), while the 95% (19/20) of pneumonia cases was due to CA-MRSA isolates.

The emergence of CA-MRSA in Uruguay has created concern regarding appropriate empirical antimicrobial therapy [7,8,22-24]. TMP-SMX and CLI have been used to treat uncomplicated SSTIs and mild invasive infections in the HP-CHPR since 2001, whereas vancomycin is reserved for life-threatening cases. However, the first two antibiotics have some limitations; for example, trimethoprim-sulfamethoxazol has no activity against group A streptococci which are sometimes involved in SSTI. Another concern is the presence of TMP-SMX resistant CA-MRSA isolates. Fortunately, we did not detect any resistance to TMP-SMX; nevertheless, a resistance rate of 97% to TMP-SMX in CA-MRSA isolates (agr type III, ST88, SCCmec IV and PVL positive) recovered from children was reported in Nigeria [25]. This group suggested that the prevalence of TMP-SMX resistance could be due to widespread and indiscriminate use of this antimicrobial combination in areas with populations of low socio-economic status and overcrowding, factors that increase the risk of microbial spreading of these strains [25]. Similar findings were reported recently in Tehran [26]. This situation may also occur in Uruguay because several of the clinical conditions are also present. It is important to maintain an active surveillance for CA-MRSA susceptibility patterns to permit early detection of changes that will affect therapeutic treatment.

Clindamycin remains a mainstay of therapy for CA-MRSA infections. However, we must take into account that isolates exhibiting the iMLS$_B$ phenotype have a high rate of spontaneous mutation that could lead to constitutive resistance; these isolates could be selected by the use of clindamycin [27-29]. In this study of 101 CA-MRSA isolates, 40 showed the iMLSB phenotype (Table 2). However, the prevalence of the iMLS$_B$ phenotype decreased over time (Figure 2) despite the increased use of clindamycin during the
study period in the HP-CHPR [30]. A similar observation was previously reported by other authors and might represent an expansion of a better adapted CA-MRSA clone lacking \textit{erm} genes as previously suggested [31].

In a previous study which included CA-MRSA strains isolated between December 2004 and November 2005 from adults living in different regions of Uruguay, we found a CA-MRSA isolate designated as group 1 (pulsotype A- \textit{USA1100}, \textit{SCCmec} IV, \textit{PVL} positive, \textit{CP} 8 and iMLSB negative) responsible for most cases of SSTI [8]. The spread and establishment of CA-MRSA isolates belonging to group 1 could also account for the situation in the paediatric population. Supporting this hypothesis, we noted that the decrease in the relative frequency of CA-MRSA isolates with iMLSB phenotype during this study occurred along with a relative increase in the proportion of CA-MRSA isolates belonging to the pulsotype A (PFGE profile type \textit{USA1100}) (Figure 3).

Six of 21 MSSA invasive isolates showed a positive result for \textit{PVL} by PCR (Table 2). Although our sample is small, the frequency of the \textit{lukS-F} genes seems relatively higher than that reported in other studies [12,32] but similar to the frequency reported by Perez-Vazquez \textit{et al.} in Spain [33]. We identified a predominant pulsotype in MSSA invasive isolates, similar to the situation observed with CA-MRSA isolates, even though the overall diversity observed within MSSA isolates was greater.

Regarding the outcome of children with CA-MRSA invasive diseases, we observed a rate of admissions to ICU (35.4%) and a mortality figure (34.7%) similar to that previously reported in the USA [21] but higher than that found in children from Tunisia [34]. However, these CA-MRSA isolates displayed microbiological characteristics similar to those recovered from children with invasive infections and better clinical outcome (Table 3). Further research on CA-MRSA pathogenesis is necessary to determine the role host factors (e.g., deficit in the generation of an oxidative burst and production of reactive oxygen species with bactericidal activity by polymorphonuclear leukocytes), other bacterial virulence traits such as enzymes and other proteins encoded in the arginine catabolic mobile element (ACME), as well as the level of gene expression of known virulence factors play in directing the differential outcome of these host-pathogen interactions.

**Acknowledgements**

We thank the Public Health Ministry for allowing us to use the CHEF II DR. We are grateful to Teresa Camou and Gabriela Garcia for their technical assistance. This study was supported by a grant from Comisión Sectorial de Investigación Científica (CSIC), Uruguay to MV.

**References**


Corresponding author
Gustavo Varela
Departamento de Bacteriología y Virologia
Alfredo Navarro 3051
PC 11600, Montevideo, Uruguay
Telephone/Fax: 598 24875795
Email: gvarela@higiene.edu.uy

Conflict of interests: No conflict of interests is declared.