Original Article

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

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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: pulsotype A (type USA1100), SCC*mec* cassette type IV, Panton-Valentine Leukocidin genes positive, susceptibility to trimethoprim-sulfamethoxazole without the inducible macrolide-lincosamide-streptogramin B ($iMLS_B$) 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

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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 SSC*mec* 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 SCC*mec* 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 ($iMLS_B$) 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 SCC*mec* 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 for quality control. A double disk diffusion test was performed to determine the $iMLS_B$ phenotype [9]. The presence of PBP2a protein in MRSA isolates was determined by commercial latex agglutination test (Oxoid).

S. aureus isolates were stored in skimmed milk frozen at -20ºC 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 SCC*mec* cassette type and presence of the *mec*A 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

	MRSA		MSSA	
Infection site	**No. of CA-MRSA Isolates [a]**	**No. of HA-MRSA Isolates [a]**	**No. of MSSA Isolates**	**TOTAL**
Bone and Joint infections	29	2	13	44
Bacteraemia	14	0	7	21
Pneumonia	19	0	1	20
CNS	2	0	0	2
Catheter infection	0	1	0	1
Endocarditis	1	0	0	1
TOTAL	65	3	21	89

[a] 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

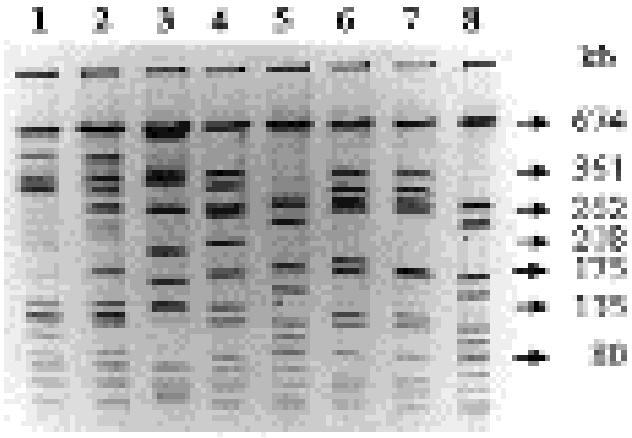
Microbiological characteristics of S. aureus *isolates recovered from invasive infections*

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 SCC*mec* 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 SCC*mec* 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 $iMLS_B$ phenotype. All 65 CA-MRSA isolates carried SCC*mec* 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 $iMLS_B$ 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



Lane 1 and 2: HA-MRSA isolates resistant to TMP-SMX; lane 3: HA-MRSA isolate susceptible to TMP-SXM (PFGE profile type "Cordobes clone"); lane 4: *S. aureus* NCTC 8325; lane 5: CA-MRSA isolate recovered from a superficial infection (PFGE profile type USA1100); lane 6 and 7: MSSA isolates recovered from invasive infections; lane 8: CA-MRSA recovered from invasive infection (PFGE profile type USA1100)

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

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

[a] One isolate carried SCC*mec* cassette type I and the other two isolates carried SCC*mec* 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

	Children with CA-MRSA invasive infections (n = 65)	
	Admitted to ICU (n = 23)	Not admitted to ICU (n = 42)
Nº (%) of children with isolates carrying the *lukS-F* genes	19 (82.6)	38 (83.3)
Nº (%) of children with isolates carrying the *cna* gene	19 (82.6)	36 (85.7)
Nº (%) of children with isolates belonging to pulsotipo A (USA1100)	19 (82.6)	33 (78.5)
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 SCC*mec* cassette. Of the 36 isolates, 29 and 30 carried the *lukS-F* and *cna* genes, respectively (Table 2). Twenty-nine strains corresponded to pulsotype 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 $iMLS_B$ phenotype (Table 2). Figure 2 shows the distribution of these 40 isolates over the years of isolation.

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 pulsotype 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

Figure 2. Evolution of the $iMLS_B$ phenotype in CA-MRSA isolates recovered from children treated at the HP-CHPR between 2003 and 2006

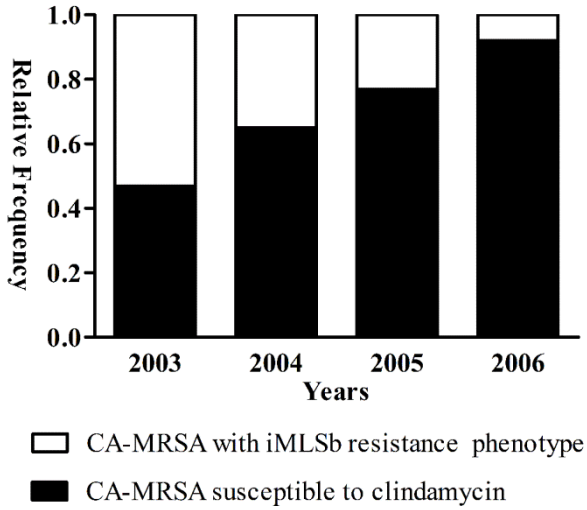
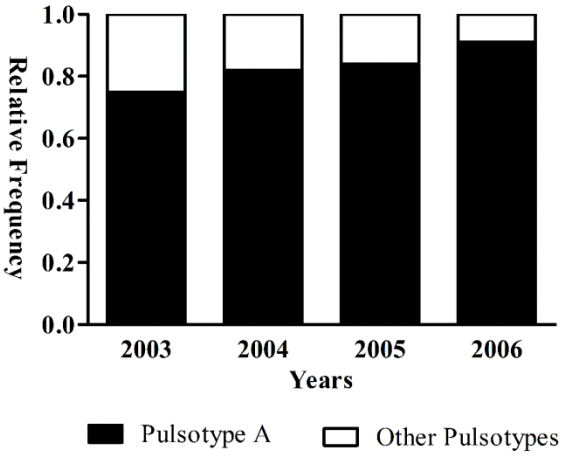


Figure 3. PFGE pulsotype distribution of CA-MRSA isolates recovered from children treated at the HP-CHPR between 2003 and 2006



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 *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- USA1100-, *SCCmec* IV, PVL positive, 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 USA1100) (Figure 3).

Six of 21 MSSA invasive isolates showed a positive result for PVL by PCR (Table 2). Although our sample is small, the frequency of the *lukS-F* genes seems relatively higher than that reported in other studies [12,32] but similar to the frequency reported by Perez-Vazquez *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.

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