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Short Communication

# Molecular epidemiology of methicillin-resistant *Staphylococcus aureus* from different population groups in Argentina

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ABSTRACT

*Objectives:* In Latin America, methicillin-resistant*Staphylococcus aureus* (MRSA) is a leading cause of nosocomial infections. Limited studies have addressed the molecular epidemiology of MRSA clones in Argentina, characterised by continuous human migratory movements. The aim of this study was to describe the MRSA epidemiology, including distinct patient populations from different regions of the country.
*Methods:* MRSA strains were collected in epidemiological studies conducted from 2009 to 2015 in three cities (Formosa, Córdoba and Tucumán) and involving four population groups: community adult patients; hospitalised adults; hospitalised children; and healthy children (nasal colonisation). Antimicrobial susceptibility testing, SCC*mec* and Panton–Valentine leukocidin (PVL) typing, pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST) were performed.
*Results:* A total of 120 MRSA isolates were recovered with an important population diversity in the groups studied; in community adult patients, MRSA isolates corresponded to ST5, ST267 and ST1619; from hospitalised adults they were ST97, ST5, ST72, ST125, ST200, ST647, ST747, ST935 and ST2941; from hospitalised children they were ST5, ST30, ST34, ST1163 and ST1619; and from colonised children they were ST5, ST125, ST34, ST100, ST1619, ST207 and ST1163. Results of SCC*mec* typing showed SCC*mec* I, SCC*mec* IIIA, SCC*mec* IV and SCC*mec* ND associated or not with PVL genes.
*Conclusions:* MRSA genetic lineages have differing distribution in the three regions. The most prevalent was ST5 in colonisation, community and invasive settings. Here we describe ST34-SCC*mec* IV clone for the first time in the hospitalised paediatric population. These findings contribute to the understanding of epidemiological changes in recent years.


## 1. Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) poses a major threat to public health worldwide owing to the rapid spread and diversification of pandemic MRSA clones with increasing virulence and antimicrobial resistance [1]. The characteristic MRSA clones circulating in south Latin American countries are the Brazilian, Cordobes/Chilean, Pediatric and New York/Japan (NY/J) clones [2–4].

In Latin America, MRSA is a leading cause of nosocomial infections and their prevalence in community-acquired infections is considerably increasing [5]. Definitively in Argentina, one-half of *S. aureus* isolates from healthcare-associated (HA) infections are MRSA (WHONET Argentina Network), with most being the Cordobes/Chilean epidemic (ST5-SCC*mec*I) HA-MRSA clone [4]. Notably, the proportion of community-associated (CA) MRSA infections in children has been increasing in the last years, and this significant increase (62%) was associated with the emergence and

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spread of the CA-MRSA clones ST5-SCC*mec*IVa-PVL$^+$ and ST30-SCC*mec*IVc-PVL$^+$ [6].

MRSA clones are genetically diverse but often present the same genes encoding antibiotic resistance, including resistance to β-lactams, erythromycin and clindamycin. In addition, most of the isolates are also resistant to rifampicin, fluoroquinolones and trimethoprim/sulfamethoxazole [7]. Multidrug resistance of MRSA clones circulating in our country limits the therapeutic options to glycopeptides, oxazolidinones and the new tetracycline derivative tigecycline, which are uniformly active [8].

MRSA carrier status has been identified as a risk factor, being a chronic reservoir for infection; in fact, MRSA clones in the nose and in different infection foci in the same patient has been demonstrated [9]. In that sense, continuous surveillance of MRSA colonisation is a challenge, particularly in patients at risk.

The Pan American Health Organization reported that resources for monitoring the changing epidemiology of MRSA in Latin American countries remain limited [10]. Significant gaps in the available data exist in Argentina, especially in local areas without extensive facilities for performing microbiological surveillance [5]. Herein we describe the current knowledge of the MRSA epidemiology, including distinct patient populations from our region, which is a multiborder area of several countries.

## 2. Materials and methods

### 2.1. Bacterial isolates

MRSA strains were collected in epidemiological studies conducted from 2009 to 2015 in three separate cities (Formosa, Córdoba and Tucumán) and involving four population groups: (i) community adult patients attending a private microbiological laboratory in Tucumán for non-invasive pathologies; (ii) admitted adult patients attending a public hospital in Tucumán for invasive infections; (iii) children (age 0–14 years) admitted to a paediatric hospital in Formosa for invasive infections; and (iv) healthy children (age 0–14 years) from Córdoba screened for *S. aureus* nasal colonisation. Each isolate corresponded to a single individual, and after isolation on trypticase soya agar the strains (one strain for each sample) were stored at −80 °C in brain–heart infusion broth with 30% glycerol (BD Diagnostics)

### 2.2. Identification and antimicrobial susceptibility testing

Strains were identified by matrix-assisted laser desorption/ionisation time-of-flight (MALDI-TOF) (Microflex LT; Bruker Daltonik GmbH, Bremen, Germany), and methicillin resistance was tested using a 30 μg cefoxitin disk (Oxoid Ltd., Basingstoke, UK). Only the MRSA isolates (cefoxitin diameter ≤22 mm) were selected. Antimicrobial susceptibility was determined by the standard agar diffusion test for erythromycin, clindamycin, gentamicin, ciprofloxacin, trimethoprim/sulfamethoxazole, rifampicin, teicoplanin, linezolid and tigecycline. Procedure recommendations and breakpoints were developed following document M100-S24 of the Clinical and Laboratory Standards Institute (CLSI). Multidrug resistance was defined as resistance to three or more antimicrobial families.

### 2.3. Staphylococcal cassette chromosome mec (SCCmec) and Panton–Valentine leukocidin (PVL) typing

SCC*mec* typing was performed by a previously described multiplex PCR protocol [11] using MRSA strains BAA-38, BAA-1681, BAA-39 and BAA-1680 as controls for SCC*mec* types I–IV, respectively. The *lukS-PV* and *lukF-PV* genes were detected using PCR primers previously described by Lina et al. [12].

### 2.4. Genetic relatedness of MRSA isolates

Molecular typing was performed by pulsed-field gel electrophoresis using *Sma*I (PFGE-*Sma*I) [13] and multilocus sequence typing (MLST) (https://pubmlst.org/saureus/). *Staphylococcus*

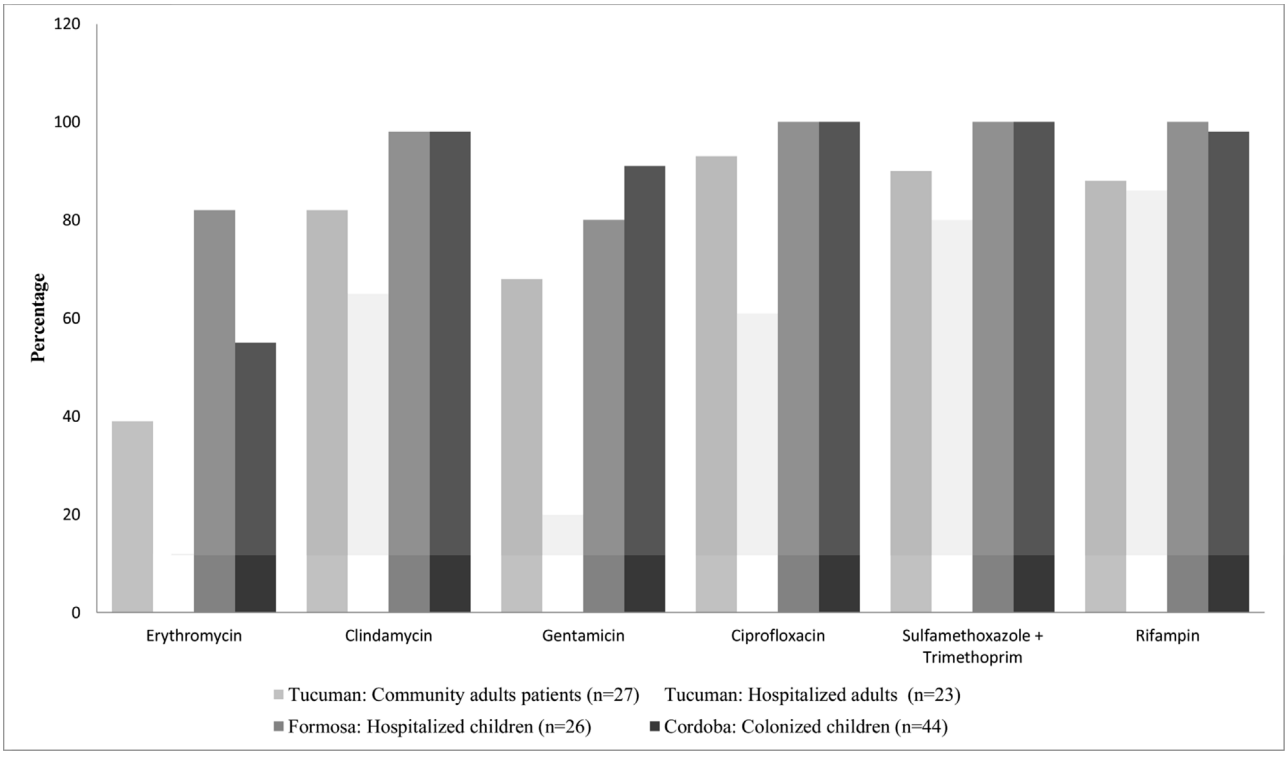


**Fig. 1.** Antimicrobial susceptibility rates detected in the methicillin-resistant *Staphylococcus aureus* (MRSA) isolates in the four populations studied.

*aureus* NCTC 8325 was used as a control in each PFGE run, and band patterns clusters were determined with Phoretix™ 5.0 software using a cut-off of 75%. Subsequently, one isolate per PFGE pulsotype was submitted to MLST. PHYLOViZ 2.0 software (http://www.phyloviz.net/) with global optimal eBURST (goeBURST) representation was used to represent the global MRSA population.

## 3. Results and discussion

A total of 120 MRSA isolates were recovered at the different samplings, including 23 isolates from community adult patients [soft tissue (39%), blood (16%), respiratory tract (16%), orthopaedic (13%), catheter (13%) and cerebrospinal fluid (3%)], 27 isolates from hospitalised adults [soft tissue (82%), orthopaedic (15%) and blood (3%)], 26 isolates from hospitalised children [soft tissue (60%), blood (30%) and orthopaedic (10%)] and 44 isolates from children with nasal carriage. In the case of nasal carriage, the 44 isolates were obtained from a total of 800 children tested (5.5% MRSA prevalence in nasal carriage).

MRSA strains presented different antimicrobial susceptibility rates according to the population group (Fig. 1), with isolates from hospitalised adults being the most resistant population. No isolate presented resistance to teicoplanin, linezolid and tigecycline.

Community adult patients were attended in a private microbiological laboratory and yielded a total of 23 isolates that were grouped in eight PFGE patterns corresponding to three sequence types (STs): ST5 was predominant, followed by ST267 and ST1619. The 27 MRSA isolates from hospitalised adults were grouped in 10 pulsotypes and nine sequence types, with ST97 being predominant and the others (ST5, ST72, ST125, ST200, ST647, ST747, ST935 and ST2941) constituting a minority (Fig. 2).

Isolates from children were divided into hospitalised, comprising 26 isolates with 10 pulsotypes and five sequence types (ST5 being the majority and the minority represented by ST30, ST34, ST1163 and ST1619), and nasal colonised, comprising 44 isolates with 14 pulsotypes and 10 sequence types (ST5, ST125 and the less represented ST34, ST100, ST1619, ST207, ST1163 and other not determined STs) (Fig. 2). PFGE analysis is not sufficient to understand the genetic structure of bacterial population as some STs contained strains belonging to different PFGE profiles.

The population diversity as well as the molecular characterisation including the SCC*mec* type and the occurrence of the PVL-encoding gene are presented in Fig. 3.

Limited studies have addressed the molecular epidemiology of MRSA clones in Argentina [8], with scarce data from our area. That was the main reason for the present work, also considering that our area is characterised by continuous human migratory movements between Chile, Bolivia and Paraguay. In the present study, the ST5-SCC*mec*IV clone predominated in the three geographical locations of the country (north, centre and northwest of Argentina) and in different population groups (community adults, infected and

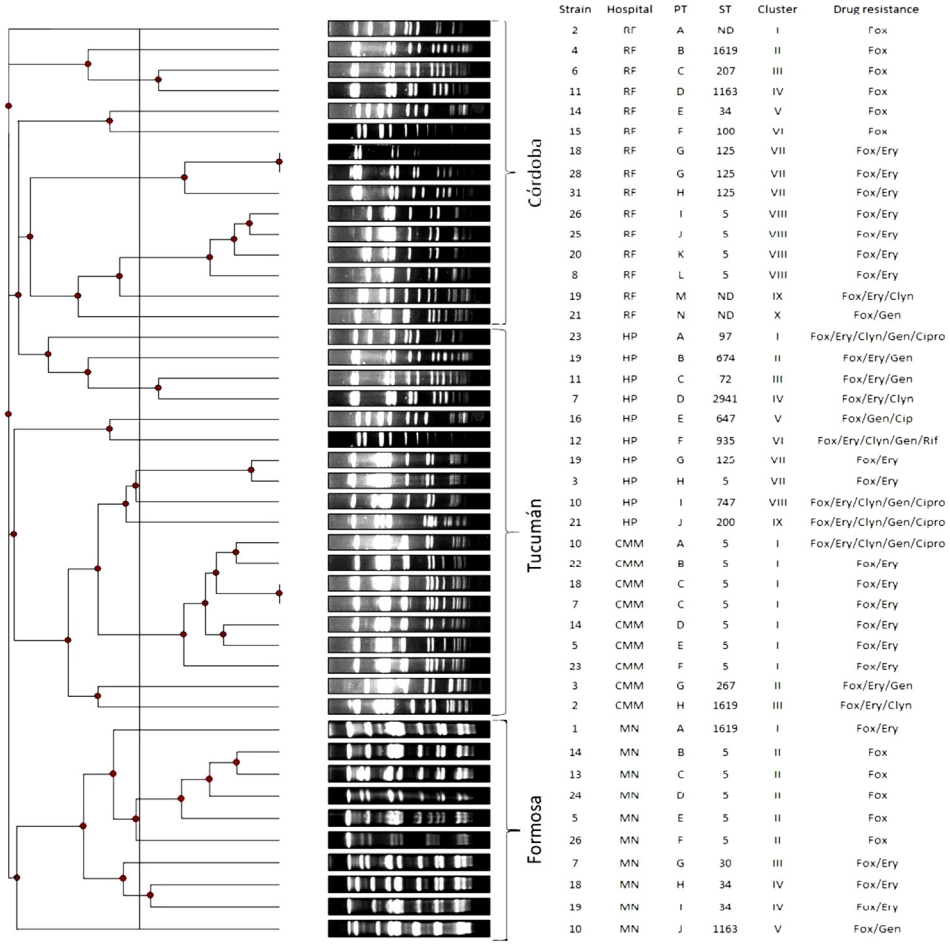


**Fig. 2.** Pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST) results of the studied methicillin-resistant *Staphylococcus aureus* (MRSA) isolates. PT, pulsotype, ST, sequence type; RF, Córdoba (colonised children); HP, Tucumán (hospitalised adults); CMM, Tucumán (community adult patients); MN, Formosa (hospitalised children); Fox, cefoxitin; Ery, erythromycin; Clyn, clindamycin; Gen, gentamicin; Cipro, ciprofloxacin; Rif, rifampicin.

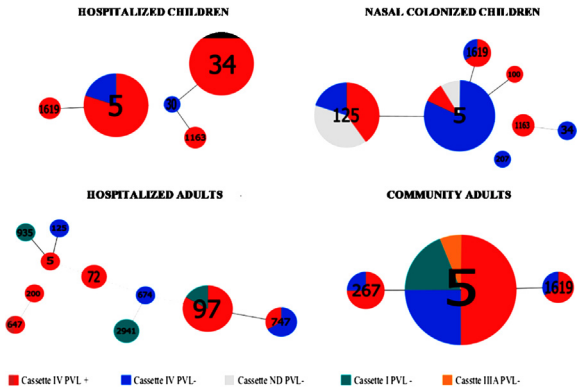


**Fig. 3.** Minimum spanning tree considering the origin and molecular characteristics of the methicillin-resistant *Staphylococcus aureus* (MRSA) isolates. 'Cassette' refers to staphylococcal cassette chromosome *mec* (SCC*mec*). PVL, Panton–Valentine leukocidin.

carrier paediatric individuals). These results differ for those reported by Sola et al. [4,6] who reported that when stratified by age the proportion of all MRSA infections caused by ST5-SCC*mec*IV MRSA clone was greater for children than for adults, with an ST30-SCC*mec*IV clone predominance in the north of the country.

The major prevalence of ST5 over ST30 in the paediatric isolates was previously described and was corroborated in our work. Sola et al. [4] reported that ST30-SCC*mec*IV and ST5-SCC*mec*IV proportions significantly differed between the north (75% and 20%) and south (18% and 55%) of the country in community settings. Subsequently, the CA-MRSA clone ST30-SCC*mec*IV has mainly spread in the north, whereas the CA-MRSA clone ST5-SCC*mec*IV has remained in the south of Argentina. These results are not in line with our results and could be the result of an epidemiological change in recent years. ST34-SCC*mec*IV clone was represented in the hospitalised paediatric collection, and curiously this clone was previously linked to maternal milk microbiota [14].

In the hospitalised adult isolate collection, a highly diverse MRSA population was observed in the absence of the Cordobes/Chilean ST5-SCC*mec*I HA-MRSA clone. This scenario is contrary to that previously described by Sola et al. Our results strongly suggest a predominance of ST5-SCC*mec*IV with an increasing reservoir in the community in carriers and that other clones appear to be replacing the classical Cordobes/Chilean clone in hospitalised adult patients. This ST5-SCC*mec*I clone has spread from the south (Argentina and Chile) to the north of Latin America through the Andean region (Perú, Ecuador, Venezuela and Colombia) as well as in Paraguay and Brazil [4,15–17]. Egea et al. [7] reported that 66% of HA-MRSA were accounted for by the CA-MRSA ST5-SCC*mec*IVa-PVL$^+$ clone, particularly in children (20% in the entire population, 46% for children). However, the ST30-SCC*mec*IVc-PVL$^+$ clone was predominant in adults (51%). In the present study it was detected only in one patient.

All these results strongly suggest that ST5-SCC*mec*IVa-PVL$^+$ is the dominant CA-MRSA clone that has entered into the hospital setting, replacing the Cordobes/Chilean HA-MRSA clone, particularly in paediatrics. This situation constitutes a concern for public health owing to the high transmissibility of this clone in the community [4,6], its great ability to cause invasive infections particularly in children, and its capacity to express the reduced susceptibility to vancomycin [vancomycin-intermediate *S. aureus* (VISA)] and heterogenous VISA (hVISA) phenotypes, as vancomycin is the mainstay of treatment [6,18]. This situation might indicate that this CA-MRSA clone is spreading not only from Uruguay [6,19] but also from other neighbouring regions to the north and northeast of Argentina, such as Brazil, where it is highly prevalent in the community [20]. As far as we know, there are no data about CA-MRSA infections in Bolivia and Paraguay. Additionally, while the Argentinean health system ensures basic coverage, access levels are particularly related to differences in the economic area of each region. According to general and health indicators, the northeast and northwest are the most impoverished and backward regions (http://www.ar.undp.org/content/argentina/es/home.html). Lower socioeconomic status (and crowded living conditions) along with lower health coverage as well as weather conditions (warmer and/or wetter) contributing to a greater density of these strains on the skin are likely important factors that might be involved in the spread of the CA-MRSA strains in the northern region of Argentina.

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## Ethical approval

Not required.

## Conflict of interests

None declared.

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