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Methicillin-resistant *Staphylococcus aureus* in hospitalized patients from the Bolivian Chaco

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SUMMARY

Objectives: Information is lacking on the methicillin-resistant *Staphylococcus aureus* (MRSA) clonal lineages circulating in Bolivia. We investigated the prevalence and molecular epidemiology of *S. aureus* colonization in hospitalized patients from the Bolivian Chaco, and compared their features with those of the few clinical isolates available from that setting.

Methods: *S. aureus* nasal/inguinal colonization was investigated in 280 inpatients from eight hospitals in two point prevalence surveys (2012, $n = 90$; 2013, $n = 190$). Molecular characterization included genotyping (*spa* typing, multilocus sequence typing, and pulsed-field gel electrophoresis), detection of virulence genes, and SCC*mec* typing.

Results: Forty-one inpatients (14.6%) were *S. aureus* nasal/inguinal carriers, of whom five were colonized by MRSA (1.8%). MRSA isolates mostly belonged to *spa*-type t701, harboured SCC*mec* IVc, and were negative for Panton–Valentine leukocidin (PVL) genes. However, a USA300-related isolate was also detected, which showed the characteristics of the USA300 Latin American variant (USA300-LV; i.e., ST8, *spa*-type t008, SCC*mec* IVc, presence of PVL genes, absence of *arc*A). Notably, all the available MRSA clinical isolates ($n = 5$, collected during 2011–2013) were also identified as USA300-LV.

Conclusions: Overall, MRSA colonization in inpatients from the Bolivian Chaco was low. However, USA300-LV-related isolates were detected in colonization and infections, emphasizing the importance of implementing control measures to limit their further dissemination in this resource-limited area.

1. Introduction

The worldwide emergence and dissemination of methicillin-resistant *Staphylococcus aureus* (MRSA) has significantly reduced the therapeutic options for staphylococcal infections and worsened their clinical outcome.[1] MRSA isolates are resistant to virtually all beta-lactams (except the newer anti-MRSA compounds) due to the expression of low-affinity penicillin-binding proteins (PBPs), encoded by the *mec*A or *mec*C genes, which can overtake the functions of the other PBPs.[1,2] The *mec* genes are carried by particular mobile genetic elements prevalent in staphylococci, named staphylococcal cassette chromosome (SCC) elements, with 11 types of SCC*mec* having been characterized so far.[2]

MRSA has been disseminating across virtually all geographical areas for decades, arising as a major pathogen in both the hospital and community setting, with a limited number of highly successful clonal lineages being responsible for most MRSA epidemics worldwide.[3] The surveillance of MRSA clones (both from infections and colonization) is crucial for the implementation of effective

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empiric treatment protocols and infection control measures, and to understand the diverse evolutionary trajectories of MRSA lineages on a worldwide scale.[3]

Latin America is not an exception to the global increasing prevalence of MRSA infections, and a number of reports have described the epidemiological and molecular features of MRSA clonal lineages circulating in this geographic area over the past two decades.[4,5] Two major MRSA clones, namely the Brazilian clone MRSA-ST239-IIIA (belonging to sequence type (ST) 239 and harbouring SCC*mec* IIIA) and the Cordobes/Chilean clone MRSA-ST5-I, have accounted for the early emergence and dissemination of MRSA in the hospital setting in several Latin American countries.[4–6] Regarding the community setting, three major MRSA lineages producing the Panton–Valentine leukocidin (PVL) have been described in Latin America: MRSA-ST30-IV and MRSA-ST5-IV, mainly disseminated in southern countries,[4,7,8] and MRSA-ST8-IVc, predominant in northern countries.[4,9–12] The latter is genetically related to the USA300 MRSA pandemic clone, but harbours a different SCC*mec* IV subtype (IVc instead of IVa) and typically lacks the arginine catabolic mobile element (ACME).[9] MRSA-ST8-IVc (sometimes referred to as USA300-LV, for USA300 Latin American variant) has recently been acknowledged as the major clone responsible for both community and hospital MRSA infections in Colombia.[11–13]

Bolivia is one of the poorest countries of Latin America, and in many rural areas the healthcare system relies on small hospitals that have no access to clinical microbiology diagnosis and limited resources for the implementation of infection control measures. An MRSA prevalence of 49% has been reported recently,[14] but data on MRSA clonal lineages circulating in this country are lacking, and very few data are available on the dissemination of MRSA in rural areas.[15,16]

In a previous surveillance study on MRSA nasal carriage, we documented a low MRSA prevalence (range 0–1.5%) among healthy individuals from the Bolivian Chaco, a resource-limited region of Bolivia.[16] In this work we investigated, for the first time, the prevalence and molecular epidemiology of *S. aureus* colonization in hospitalized patients from that region, and compared their features with those of the few *S. aureus* clinical isolates available from that setting.

2. Methods

2.1. Study design and population

S. aureus colonization was investigated in eight hospitals in seven small urban areas of the Bolivian Chaco region (Figure 1 and **Supplementary Material** Table S1). All hospitals are small healthcare units with 20 to 78 beds (**Supplementary Material** Table S1), and together are representative of the organization of the hospital care system in this area. Facilities for microbiological diagnosis of skin and soft tissue infections (SSTIs) were not available at these hospitals, with the exception of one of them – the hospital of Villa Montes. This hospital has performed microbiological analyses since mid-2010, although a very limited number of samples are processed.

The survey was a point prevalence study performed twice (on August 2–3, 2012, and on August 12–17, 2013). All individuals hospitalized during the study periods were considered eligible. After providing written informed consent (obtained from the parents or legal guardians in the case of a minor), samples were obtained from each patient for the detection of *S. aureus* colonization (see below). Full ethical clearance was obtained from the qualified authorities who revised and approved the study design (Convenio de Salud, Ministerio de Salud – Vicariato de Camiri, Camiri, Bolivia).

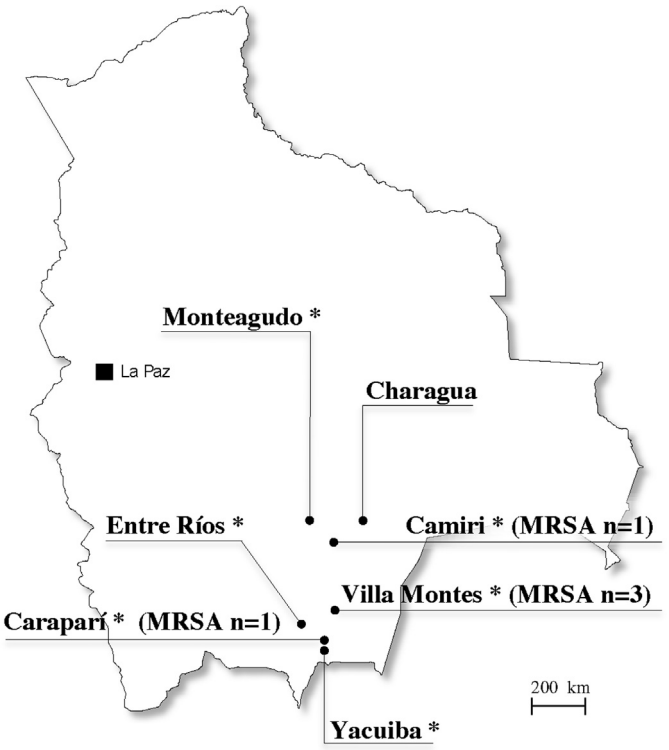

Figure 1. Location of the centres involved in the study. The study involved eight hospitals: Monteagudo (Hospital San Antonio de Los Sauces, Hospital Dermatologico); Charagua (Hospital Mamerto Eguez Soruco); Camiri (Hospital Municipal de Camiri); Villa Montes (Hospital de Villa Montes); Caraparí (Hospital de Caraparí); Entre Rios (Hospital de Entre Rios); Yacuiba (Hospital de Yacuiba). Hospitals with patients colonized by *Staphylococcus aureus* are marked with an asterisk and the number of patients carrying MRSA is reported in brackets.

For comparison purposes, all *S. aureus* clinical isolates collected in the hospital of Villa Montes since the introduction of the clinical microbiology laboratory were included in the present study (see below).

2.2. Screening for carriage of S. aureus

The investigation of *S. aureus* colonization was performed by obtaining two samples from each participant: a nasal swab (a single swab for both nares) and an inguinal swab (a single swab for both groin sides). The nasal and inguinal swabs obtained from each individual were preserved at 4 °C in Amies transport medium (Oxoid, Milan, Italy) and transported to the hospital of Camiri, where the swabs were processed as follows. Each pair of swabs (from each subject) was inoculated overnight at 35 °C in an enrichment medium (2 ml) constituted of tryptic soy broth (TSB) (Oxoid) supplemented with 6.5% NaCl and 25 μg/ml colistin (prepared by adding 1 disk of colistin 25 μg per millilitre of broth). Then, 10 μl of the enriched suspension was plated onto mannitol salt agar (MSA) (Oxoid); the bacterial growth was collected and preserved in Amies transport medium for transfer to Italy. Here, each sample was again plated on MSA, and mannitol-fermenting colonies were subcultured and identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS system; bioMérieux Inc., Marcy l'Etoile, France). For each sample, only one *S. aureus* isolate was selected for further analysis.

2.3. In vitro susceptibility testing

Antimicrobial susceptibility testing was performed by disk diffusion method in accordance with the Clinical and Laboratory