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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).

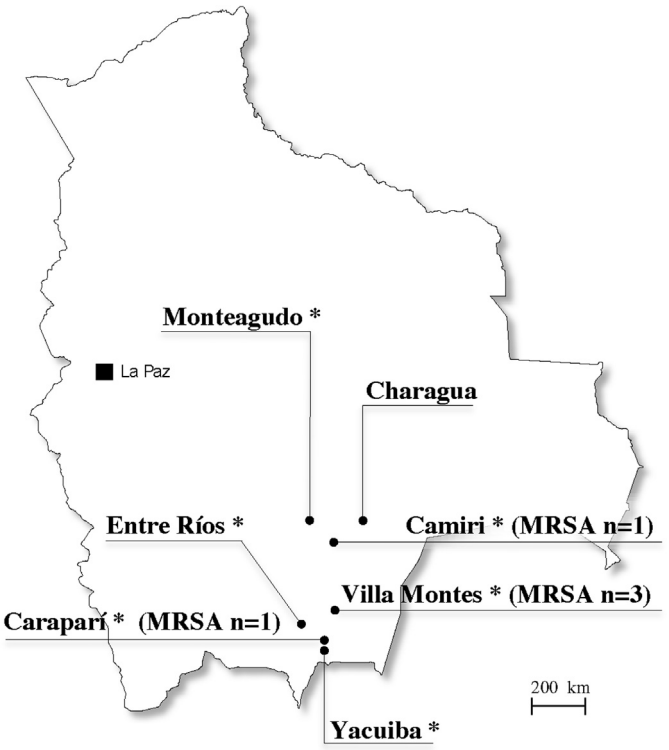

Figure 1. Location of the centres involved in the study. The study involved eight hospitals: Monteagudo (Hospital San Antonio de Los Sauces, Hospital Dermatologico); Charagua (Hospital Mamerto Eguez Soruco); Camiri (Hospital Municipal de Camiri); Villa Montes (Hospital de Villa Montes); Caraparí (Hospital 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

Standards Institute (CLSI) guidelines.[17,18] Antibiotic disks were purchased from Oxoid. All isolates were tested for susceptibility to penicillin G, erythromycin, clindamycin, tetracycline, ciprofloxacin, trimethoprim–sulfamethoxazole, gentamicin, chloramphenicol, and rifampin. In addition, they were investigated for the methicillin resistance phenotype by cefoxitin screening disk test, and for inducible clindamycin resistance by D-zone test.[18] *S. aureus* ATCC 25923 was used as quality control.

2.4. Molecular characterization of S. aureus isolates

PVL genes and the *arc*A gene (for ACME) were detected by PCR, as described previously.[10] Genotyping was performed by *spa* typing,[19] multilocus sequence typing (MLST) (http://www.mlst.net),[20] and pulsed-field gel electrophoresis (PFGE) following the CDC protocol (http://www.cdc.gov/HAI/pdfs/labSettings/ar_mras_PFGE_s_aureus.pdf) and interpreted according to the criteria proposed by van Belkum et al.[21] SCC*mec* characterization was achieved following the PCR-based protocols described previously by Kondo et al. and Milheiriço et al. for typing and subtyping, respectively.[22,23]

2.5. Clinical isolates

Clinical isolates were collected in the hospital of Villa Montes, the only one with facilities for the microbiological diagnosis of SSTIs. During the period May 2010 to August 2013, only 10 *S. aureus* clinical isolates were collected from that hospital. This limited number is related to the fact that microbiological diagnosis represents an extra cost for the patient and is rarely requested by physicians. Of the 10 isolates, only nine were available for investigation. Identification was confirmed by MALDI-TOF MS (bioMérieux) and characterization was carried out as described for *S. aureus* isolates from colonization.

2.6. Statistical analysis

Statistical differences were determined by Chi-square test (with Yates' correction) and Fisher's exact test when appropriate. Confidence intervals (95% CI) were calculated using the binomial distribution.

3. Results and discussion

3.1. Characteristics of the study population

Among all individuals admitted to hospital during the point prevalence study periods, 280 (2012, $n = 90$; 2013, $n = 190$) agreed to participate in the survey. In 2013, 80% of inpatients consented to sampling, while no data were available for the 2012 survey.

The male to female ratio of the study participants was 181:99, and they ranged in age from 1 day to 89 years (mean age 31 years and median age 26 years; calculated from all but five patients, for whom age was not available). Inpatients were admitted to maternity ($n = 93$), general medicine ($n = 78$), surgery ($n = 50$), paediatric ($n = 45$), tuberculosis isolation ($n = 7$), first aid ($n = 4$), and intensive care ($n = 3$) wards. At the time of sample collection, 63% of patients had been hospitalized within the previous 48 h and 77% of them reported no hospitalization during the preceding year.

3.2. Colonization by S. aureus among inpatients from eight hospitals in the Bolivian Chaco region

An overall moderate prevalence of *S. aureus* colonization was detected among the 280 hospitalized individuals included in the study ($n = 41$, 14.6%), with no significant differences observed between the two study periods (17.8% (95% CI 10–27%) and 13.2% (95% CI 9–19%) in 2012 and 2013, respectively; $p = 0.4$). Carriers of *S. aureus* were found in all hospitals except in Charagua (where nine inpatients were enrolled in 2012 and 15 in 2013) (Figure 1 and **Supplementary Material** Table S1).

Colonization by MRSA isolates was detected with a rate of 1.1% (95% CI 0–6%) in 2012 and 2.1% (95% CI 0–5%) in 2013 ($p = 1$), and involved five inpatients from three out of the eight hospitals (Camiri, $n = 1$; Caraparí, $n = 1$; Villa Montes, $n = 3$) (Figure 1 and **Supplementary Material** Table S1). No relevant correlation between methicillin-sensitive *S. aureus* (MSSA)/MRSA carriage and clinical/demographic data was observed (data not shown). The observed MRSA colonization rate (1.8% of the total studied population) was overall consistent with the results of a previous study conducted in the same geographic region in 2008 and 2009, which revealed MRSA colonization in three out of 196 (1.5%) healthy individuals from a rural village.[16] A similar MRSA colonization rate was also reported recently in a population-based study performed in a small city of Brazil (0.9%) and among medical students in Colombia (1.6%).[24,25] The particular healthcare system of the Bolivian Chaco, based on very small hospitals, and the fact that 63% of samples were collected within 48 h of admission, could explain the similar MRSA colonization rates observed in the community and hospital settings in this region.

3.3. Characterization of S. aureus isolates from carriers

Susceptibility testing revealed overall low resistance rates in *S. aureus* isolates from carriers. Among MSSA isolates ($n = 36$), most (83%) were non-susceptible to penicillin G, while only a few were non-susceptible to tetracycline (14%), gentamicin (8%), chloramphenicol (6%), and erythromycin (3%, with inducible clindamycin resistance), and all isolates were susceptible to trimethoprim–sulfamethoxazole, ciprofloxacin, linezolid, and rifampin. MRSA isolates ($n = 5$) were susceptible to all of the above non-beta-lactam antibiotics tested (except for one isolate that was non-susceptible to erythromycin and ciprofloxacin).

MSSA isolates tested negative for *mec*A and PVL genes, while all MRSA isolates were found to carry SCC*mec* IVc, and one of them was PLV-positive. *spa* typing analysis revealed a high heterogeneity among MSSA isolates (20 different types, including the new *spa*-type t13417), without a predominance of any *spa*-type (Table 1). In contrast, the five MRSA isolates were found to belong to two *spa*-types: t701 (one isolate in 2012 and three in 2013) and t008 (one isolate in 2013) (Table 1).

Interestingly, besides being the dominant *spa*-type among MRSA isolates in this survey (Table 1), *spa*-type t701 was also found in three MSSA isolates from this survey and all of the MRSA isolates ($n = 3$) detected in a previous study on MRSA nasal carriage in a small rural community in the same geographical setting (Table 2).[16] In order to investigate their clonal relationships, the seven MRSA and three MSSA isolates belonging to t701 were subjected to PFGE analysis, which assigned all isolates to the same clonal lineage (PFGE type A) with a maximum of five different bands (Table 2). These data demonstrate that *spa*-type t701 is endemic in this area (being detected over a period of 5 years in the microbiota of both healthy individuals and inpatients), and suggest the likely local evolution of MRSA from MSSA (or vice versa), as described in other settings.[26] MRSA and MSSA belonging to *spa*-type t701 are described all over the world, from infection and colonization, as also reported in the Ridom SpaServer (http://www.spaserver.ridom.de/). In addition, a recent publication reported that t701 (harbouring different SCC*mec* types) accounted for 30% of MRSA clinical isolates from diverse hospitals in the west of Iran,[27] suggesting a propensity of this lineage to epidemic dissemination.

Table 1
Population structure of *Staphylococcus aureus* isolates from colonization and infection in patients from eight hospitals in the Bolivian Chaco

Source	Year	MSSA/ MRSA	Number of isolates	*Spa*-type (number of isolates)
Colonization	2012	MSSA	15	t189 (*n* = 3); t701 (*n* = 3); t359 (*n* = 2); t2883 (*n* = 2); t002 (*n* = 1); t088 (*n* = 1); t645 (*n* = 1); t1671 (*n* = 1); t6907 (*n* = 1)
		MRSA	1	t701 (*n* = 1)
Colonization	2013	MSSA	21	t024 (*n* = 3); t189 (*n* = 3); t078 (*n* = 2); t645 (*n* = 2); t729 (*n* = 2); t065 (*n* = 1); t319 (*n* = 1); t1166 (*n* = 1); t1451 (*n* = 1); t4710 (*n* = 1); t5365 (*n* = 1); t6125 (*n* = 1); t13417 (*n* = 2)
		MRSA	4	t701 (*n* = 3); t008 (*n* = 1)
Infection[a]	2010–2013	MSSA	4	t002 (*n* = 1); t021 (*n* = 1); t088 (*n* = 1); t645 (*n* = 1)
		MRSA	5	t008 (*n* = 5)

MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-sensitive *Staphylococcus aureus*.
[a] All clinical isolates except three (t645, t088, and one representative of t008) were from inpatients.

Table 2
Features of *Staphylococcus aureus* isolates from colonization and infection belonging to *spa*-types t701 and t008

Source	Year	Isolate	Origin	Type (population)	MRSA/MSSA	*spa*-type	PVL	SCC*mec*	PFGE[c]
Colonization	2012	131a	Yacuiba	Hospital	MSSA	t701	Neg	NA	A_3
		176a	Monteagudo	Hospital	MSSA	t701	Neg	NA	A_3
		188a	Villa Montes	Hospital	MSSA	t701	Neg	NA	A_2
		121a	Camiri	Hospital	MRSA	t701	Neg	IVc	A_1
Colonization	2013	272a	Villa Montes	Hospital	MRSA	t701	Neg	IVc	A
		280a	Villa Montes	Hospital	MRSA	t701	Neg	IVc	A
		281a	Villa Montes	Hospital	MRSA	t701	Neg	IVc	A
		284a	Caraparí	Hospital	MRSA[d]	t008	Pos	IVc	B
Infection[a]	2010–2013	304	Villa Montes	Hospital	MRSA	t008	Pos	IVc	B
		306	Villa Montes	Hospital	MRSA	t008	Pos	IVc	B
		233	Villa Montes	Hospital	MRSA	t008	Pos	IVc	B
		393	Villa Montes	Hospital	MRSA	t008	Pos	IVc	B_1
		401	Villa Montes	Hospital	MRSA	t008	Pos	IVc	B
Colonization[b]		140	Gutierrez	Community	MRSA	t701	Neg	IVc	A
	2009	99	Gutierrez	Community	MRSA	t701	Neg	IVc	A_1
		132	Gutierrez	Community	MRSA	t701	Neg	IVc	A_1

MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-sensitive *Staphylococcus aureus*; PVL, Panton–Valentine leukocidin; SCC*mec*, staphylococcal cassette chromosome *mec*; PFGE, pulsed-field gel electrophoresis; NA, not applicable.
[a] All isolates belonging to *spa*-type t008 were from inpatients, with the exception of isolate 304, which was from an outpatient. Isolates 304 and 306 were collected in 2011, isolate 233 in 2012, and isolates 393 and 401 in 2013.
[b] Previously characterized by Bartoloni et al., 2013.[16]
[c] Two different bands (A/A_1, A_2/A_3); three different bands (A/A_2); five different bands (A/A_3, A_1/A_2, A_1/A_3).
[d] All MRSA isolates remained susceptible to all non-beta-lactam antibiotics tested, with the exception of isolate 284a, which showed intermediate susceptibility to erythromycin and ciprofloxacin.

The MRSA isolate belonging to *spa*-type t008 was the only one testing positive for PVL genes and showing non-susceptibility to erythromycin and ciprofloxacin (Table 2). MLST assigned it to ST8 (ST8 clonal complex). As ST8, *spa*-type t008, SCC*mec* IVc, and PLV positivity are among the features of USA300 Latin American variant (USA300-LV), we also tested this isolate for the presence of ACME. The absence of ACME confirmed that this isolate was related to USA300-LV, one of the most widespread MRSA clones and largely dominant in community and hospital settings in northern countries of Latin America.[4,9–13]

3.4. Comparison between S. aureus isolates from colonization and infection

In order to compare *S. aureus* isolates from colonization and infection, nine clinical isolates collected from SSTIs in Villa Montes were also analyzed. They represented all of the *S. aureus* isolates collected in that hospital during the period May 2010 to August 2013, with the exception of one isolate that had not been stored.

Four of them (44.4%) were MSSA, showed a susceptibility phenotype to non-beta-lactam antibiotics, and were negative for the presence of PVL genes. The remaining five isolates (55.6%) were MRSA and were found to share the same features as the MRSA isolate from colonization belonging to *spa*-type t008. Indeed, they were all assigned to *spa*-type t008, showed a similar PFGE pattern (PFGE type B), carried SCC*mec* IVc and PVL genes, and were negative for the presence of ACME. However, differently from the isolate from colonization, they remained susceptible to all non-beta-lactam antibiotics tested.

The low number of *S. aureus* clinical isolates available for investigation and the possibility of obtaining clinical isolates from only one hospital are limitations of the present work. Nonetheless, USA300-LV was isolated over a 3-year period (i.e., first isolate on January 2011, last isolate on June 2013) and accounted for all documented MRSA infections. The identification of USA300-LV clinical isolates in the hospital of Villa Montes over an almost 3-year period, together with the detection of one USA300-LV carrier in a hospital from another urban area, would suggest the dissemination of this relevant clone in the Chaco region.

4. Conclusions

MRSA is a global public health threat in both the hospital setting and in the community.[1]

In this study, we surveyed colonization by *S. aureus* in patients from eight hospitals in the Bolivian Chaco, which is a distinct setting because of the presence of very small hospitals (representative of the local healthcare system), the absence of facilities for microbiological diagnosis, and as a consequence, a lack of microbiological data.

In this context, not studied before, we found a moderate (14.6%) *S. aureus* carriage, comparable to data reported previously

worldwide,[28] and an overall low rate (1.8%) of MRSA carriers. Isolates belonging to *spa*-type t701 seemed to be the most prevalent among MRSA, being disseminated in both the hospital and community setting, but a USA300-LV isolate was also detected. Of note, representatives of this clone were the only type of MRSA detected among clinical isolates of *S. aureus* available for investigation from the study area, underscoring the clinical and epidemiological impact of this clone even in this setting.[9]

Overall, these findings underscore the importance of implementing infection control measures in similar settings and may suggest that alternatives to beta-lactams be considered when empiric antimicrobial therapy is provided for the treatment of an infection compatible with *S. aureus* aetiology. All MRSA isolates were found to be susceptible to several antimicrobials including first-line, oral, and inexpensive drugs such as trimethoprim–sulfamethoxazole and tetracycline. As the prevalence of resistance to non-beta-lactam agents could change over time, susceptibility patterns of *S. aureus* should continue to be monitored, and the information used to guide empiric management decisions.

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Ethical approval: 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).

Conflict of interest: No conflict of interest to declare.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ijid.2014.12.006.

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