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Low prevalence of methicillin-resistant *Staphylococcus aureus* nasal carriage in urban and rural community settings in Bolivia and Peru☆

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SUMMARY

Objective: To investigate the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) nasal carriage in rural and urban community settings of Bolivia and Peru.
Methods: MRSA nasal carriage was investigated in 585 individuals living in rural and urban areas of Bolivia and Peru (one urban area, one small rural village, and two native communities, one of which was highly isolated). MRSA isolates were subjected to molecular analysis for the detection of virulence genes, characterization of the staphylococcal cassette chromosome *mec* (SCC*mec*), and genotyping (multilocus sequence typing (MLST) and pulsed-field gel electrophoresis (PFGE)).
Results: An overall very low prevalence of MRSA nasal carriage was observed (0.5%), with MRSA carriers being detected only in a small rural village of the Bolivian Chaco. The three MRSA isolates showed the characteristics of community-associated MRSA (being susceptible to all non-beta-lactam antibiotics and harboring the SCC*mec* type IV), were clonally related, and belonged to ST1649.
Conclusions: This study provides an insight into the epidemiology of MRSA in community settings of Bolivia and Peru. Reliable, time-saving, and low-cost methods should be implemented to encourage continued surveillance of MRSA dissemination in resource-limited countries.

1. Introduction

Staphylococcus aureus can cause a wide range of human diseases, being among the major human pathogens both in the community and in the hospital setting.[1] In fact, it is a leading cause of complicated skin and soft tissue infections, as well as of serious nosocomial infections, such as bloodstream infections, pneumonia, and other biofilm-associated difficult-to-treat infections.[1] *S. aureus* is also a common colonizer of the human host and can be detected in the nares and at other body sites (e.g., pharynx, groin, axillae, vagina, and gastrointestinal tract) in a large proportion of healthy individuals (approximately 30%, with important differences in carriage rates depending on the studied population).[2] Colonization is known to represent an important risk factor for infection.[1,2]

Over the past few decades, *S. aureus* has become an even greater therapeutic challenge following the emergence of methicillin-resistant *S. aureus* (MRSA) strains, which are resistant to all available beta-lactams due to the production of the low affinity penicillin-binding protein PBP2a (encoded by the transferable *mecA* gene).[3] MRSA strains were confined for a long time to hospitals and other health care settings (hospital-associated MRSA, HA-MRSA), but have recently also emerged as community-associated pathogens (CA-MRSA), generating an additional public health concern.[3] Relatively few clonal lineages are responsible for the majority of MRSA infections worldwide. In fact, evolutionary

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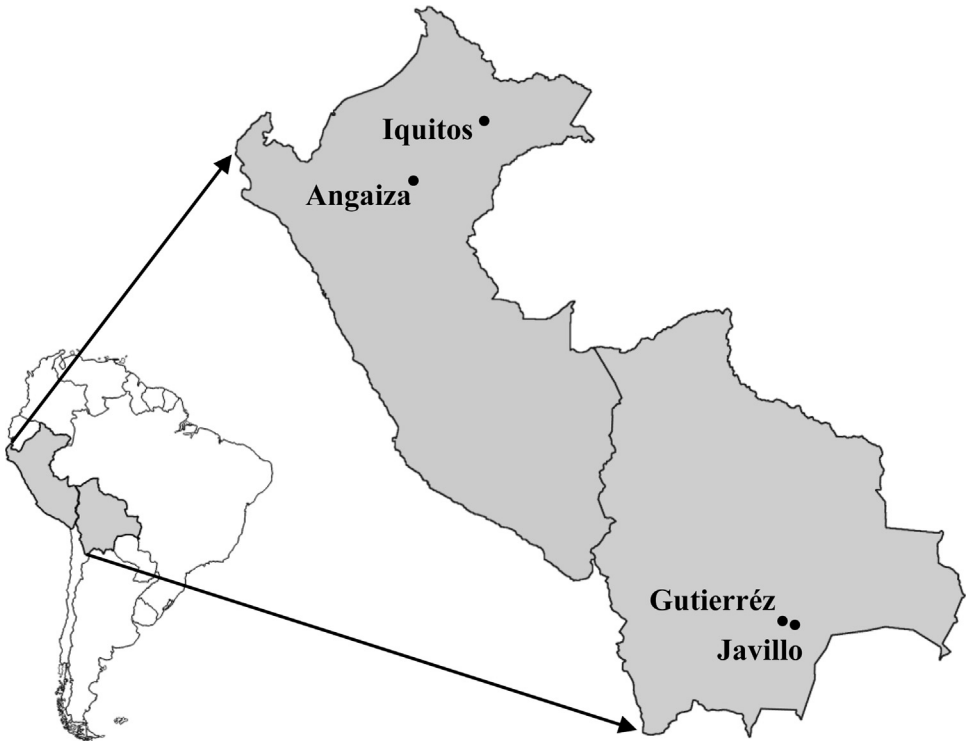

Figure 1. Studied communities.

studies have shown that MRSA evolved through the acquisition of *mecA* by already epidemic methicillin-susceptible *S. aureus* clones, giving rise to five major pandemic lineages that have spread globally (clonal complexes CC5, CC8, CC2, CC45, and CC30).[4]

Latin America is not an exception in the worldwide increasing incidence of MRSA infections, although the molecular epidemiology of MRSA clones in this area is still largely unknown, especially in the community setting.[5]

Here we report the results of a study on the prevalence and molecular epidemiology of MRSA nasal carriage in rural and urban community settings of Bolivia and Peru.

2. Materials and methods

2.1. Study design and population

MRSA nasal carriage was investigated in 585 individuals living in rural and urban areas of Bolivia and Peru (Figure 1). The study was conducted in an urban area of Peru (Iquitos, Loreto Department), in a small rural village of Bolivia (Gutierrez, Santa Cruz Department), and in two native communities including a Guaraní community located in the Bolivian Chaco (Javillo, Santa Cruz Department) in regular contact with the village of Gutierrez, and a very remote and highly isolated Chayahuita community in the Peruvian Amazon forest (Angaiza, Loreto Department) (Figure 1 and Table 1). In Iquitos and Gutierrez the studied households were selected with a modified cluster sampling,[6] and criteria for eligibility of individuals within each selected household were the following: up to four individuals (two younger and two older than 14 years) in Iquitos, and all available individuals in Gutierrez (Table 1). In the native communities, all available individuals were considered eligible for the study (Table 1). Prior to enrollment, written informed consent was obtained from all adult participants and from the parents or legal guardians of minors. Any literate participant signed the consent form. In the case of an illiterate participant, the consent form was read and signed by a witness (who was present throughout the consent procedure and interview) and marked by the participant/parent. The consent procedure and interviews were always conducted by trained local healthcare workers (with the help of a local translator in the native communities). In each country, full ethical clearance was obtained from the qualified authorities who had revised and approved the study design (Convenio de Salud, Ministerio de Salud – Vicariato de Camiri, Camiri, Bolivia; Comité Institucional de Ética de la Universidad Peruana Cayetano Heredia, Lima, Peru).

2.2. Screening for MRSA nasal carriage

For each individual included in the study, nasal swabs were obtained from both nares (using a single swab); these were preserved in Amies transport medium (Oxoid, Italy) and processed within 4 days. Each swab was eluted in 0.5 ml of normal saline (room temperature for 10 min), and 10 μl of the suspension were plated onto mannitol salt agar (MSA), placing a cefoxitin disk

Table 1
Characteristics of the studied communities, sampling criteria, and prevalence of MRSA nasal carriers

Setting	Type (population)	Year	Sampling criterion	Tested individuals	Carriers, *n* (%)
Javillo, Bolivia	Native community (ca. 100)	2008	All available individuals	64	0 (0)
Gutierrez, Bolivia	Rural village (ca. 700)	2009	Random sampling	196	3 (1.5)
Iquitos, Peru	Urban area (ca. 400 000)	2009	Random sampling	200	0 (0)
Angaiza, Peru	Remote native community (ca. 130)	2009	All available individuals	125	0 (0)

(Oxoid) onto the most heavily inoculated area. Colonies that grew within the inhibition zone were subcultured onto MRSA chromogenic agar (Oxoid), and one colony of putative MRSA from each sample was further studied. *S. aureus* species identification was confirmed by detection of coagulase activity (using rabbit plasma), by the Vitek-2 system (bioMérieux Inc., Marcy l'Etoile, France), and by a PCR approach using primers targeting the *eap* gene.[7]

2.3. In vitro susceptibility testing

Antimicrobial susceptibility was determined by the disk diffusion method, in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines.[8,9] Antibiotics tested included cefoxitin, penicillin G, erythromycin, clindamycin, tetracycline, ciprofloxacin, trimethoprim–sulfamethoxazole, gentamicin, chloramphenicol, and rifampin (Oxoid). Inducible clindamycin resistance was investigated by the D-zone test, as per the CLSI guidelines. *S. aureus* ATCC 25923 was used for quality control purposes. Methicillin resistance was always confirmed by amplification of the *mecA* gene, as described previously.[10]

2.4. Characterization of MRSA strains

All MRSA isolates were subjected to molecular analysis for the detection of virulence genes, characterization of the staphylococcal cassette chromosome *mec* (SCC*mec*), and genotyping.

Detection of the Panton–Valentine leukocidin (PVL) was performed by PCR amplification of the *lukS-PV* and *lukF-PV* genes, as proposed by Lina et al.[11] SCC*mec* typing (including subtyping of SCC*mec* type IV) was performed by previously described multiplex PCR approaches.[10,12] Multilocus sequence typing (MLST) was performed as described by Enright et al.,[13] with analysis of sequences held on the MLST website (http://www.mlst.net). Pulsed-field gel electrophoresis (PFGE) was carried out using the standard US Centers for Disease Control and Prevention (CDC) PulseNet protocol available from the CDC website (http://www.cdc.gov/HAI/pdfs/labSettings/ar_mras_PFGE_s_aureus.pdf).

3. Results

3.1. Prevalence of MRSA nasal carriage

In the present study, an overall very low prevalence of MRSA nasal carriage was observed among apparently healthy individuals living in urban and rural settings of Bolivia and Peru (Table 1). In fact, of the 585 individuals studied, only three (0.5%) were found to be colonized by MRSA. MRSA carriers were only detected in the small rural village of the Bolivian Chaco (three of 196 individuals investigated, 1.5%), whilst carriage of MRSA was not observed in the two native communities nor, quite unexpectedly, in the urban area in Peru (Figure 1).

MRSA carriers from Gutierrez (aged 51, 11, and 11 years, respectively) were found to belong to three different households, and one of them reported hospitalization during the 12 months preceding the study (an 11-year old child). Recent antibiotic use (i.e., 2 weeks preceding the study) was excluded for all of them. Other studied members of these three households (n = 10, 6, and 8, respectively) tested negative for MRSA colonization, and none of them reported a recent hospitalization.

3.2. Characterization of MRSA strains

The three MRSA isolates were found to be susceptible to other antibiotics tested including erythromycin, clindamycin, tetracycline, ciprofloxacin, trimethoprim–sulfamethoxazole, gentamicin, chloramphenicol, and rifampin.

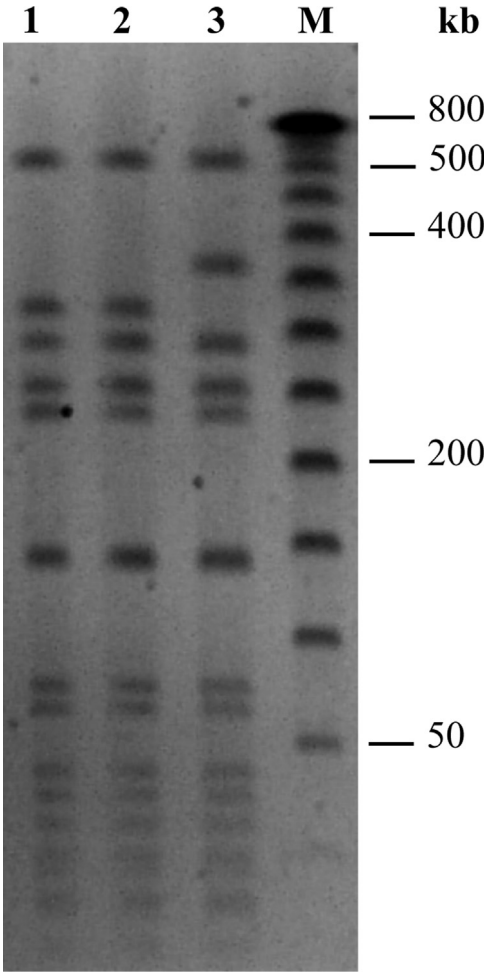

Figure 2. PFGE patterns of the three MRSA isolates from Gutierrez, Bolivia. M, ProMega-Markers Lambda Ladders (Promega, Madison, WI), with DNA size standards indicated on the right in kilobase pairs.

Molecular analysis revealed that they belonged to ST1649 and exhibited related PFGE profiles (i.e., two were identical and one differed for 2 bands) (Figure 2). All harbored SCC*mec* type IV (subtype c or e, as per the Milheiriço scheme)[12] and were PVL-negative.

4. Discussion

MRSA clones have emerged as a global public health concern both in the hospital and in the community setting.[1,3] Despite one of the five major MRSA pandemic clones originating in Brazil (the so-called Brazilian clone) and several epidemic MRSA clones having spread through Latin America in the last two decades, data on the molecular epidemiology of MRSA in this area remain limited, especially those concerning the community setting.[5]

In the present study, we documented that nasal carriage of MRSA among apparently healthy individuals from urban and rural settings of Bolivia and Peru remains very low (range 0–1.5%). To the best of our knowledge, this is the first study on the prevalence of MRSA nasal carriage in the community setting in these two Latin American countries.

The three MRSA isolates detected in this study (all from the small Bolivian village) showed the characteristics of CA-MRSA (being susceptible to all non-beta-lactam antibiotics and harboring the SCC*mec* type IV), were clonally related, and were assigned to ST1649 (belonging to CC6). MRSA isolates belonging to ST1649 or

CC6 have already been detected sporadically in nasal specimens from healthy individuals in European countries.[14,15]

Efforts should be made to implement reliable, time-saving, and low-cost methods for the detection of MRSA colonization in order to encourage continued surveillance of MRSA dissemination in resource-limited countries.

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Ethical approval: In each country, full ethical clearance was obtained from the qualified authorities who had revised and approved the study design (Convenio de Salud, Ministerio de Salud – Vicariato de Camiri, Camiri, Bolivia; Comité Institucional de Ética de la Universidad Peruana Cayetano Heredia, Lima, Peru).

Conflict of interest: No conflict of interest to declare.

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