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Antimicrobial resistance and molecular typing of *Staphylococcus aureus* bloodstream isolates from hospitals in Peru

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Summary *Objectives:* Methicillin-resistant *Staphylococcus aureus* (MRSA) infections are of worldwide concern. The present study describes the antimicrobial resistance and molecular typing of methicillin-resistant and methicillin-susceptible *S. aureus* (MSSA) bloodstream isolates in Peru.
Methods: Consecutive non-duplicate *S. aureus* bloodstream isolates were collected over a 15-month period (2008—2009) from seven hospitals in Lima and Callao, two contiguous cities in Peru. Detection of *mec*A gene, *spa* typing and Staphylococcal Chromosomal Cassette (SCC) *mec* typing were performed. Antimicrobial resistance was assessed by disk diffusion.
Results: Of 338 isolates, MRSA rate was 50.0%. Among MRSA isolates ($n = 169$), 81.7% were associated to MLST CC5, 68.8% had *spa* t149/SCC*mec* I, and more than 85% were co-resistant to ciprofloxacin, clindamycin, erythromycin and gentamicin; 8.9% ($n = 15$) were associated to MLST CC8, 14 of them had *spa* t148/SCC*mec* IV, and more than 70% were co-resistant to ciprofloxacin, clindamycin and erythromycin. Among MSSA isolates ($n = 169$), there was a higher diversity of *spa* types ($n = 56$) compared to MRSA isolates ($n = 17$), 27.2% were associated to MLST CC8, 23.7% were resistant to erythromycin and clindamycin resistance exceeded 20%.
Conclusions: MRSA rate among bloodstream isolates in Peru was 50%, with MLST CC5/t149/SCC*mec* I representing the most frequent clone.

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Introduction

Staphylococcus aureus causes serious infections and methicillin-resistant *S. aureus* (MRSA) is associated with a two-fold higher mortality rate compared to methicillin-susceptible *S. aureus* (MSSA).[1] The MRSA rates reported in the vast region of Latin America are very variable and range from 6% in Cuba to 80% in Chile.[2] The frequency of MRSA infections has been increasing in Latin America, from 33.8% in 1997 to 40.2% in 2006 and there has also been an increase in the resistance rates to most other antimicrobials.[3] As to the genetic profile of MRSA, there are five MRSA predominant clones circulating in Latin America. The Brazilian clone was first described in 1994 and was followed by the description of the Chilean (2001), the Pediatric (2002), the Cordobes (2002), and the New York/Japan (2004).[4–8] The Cordobes and Chilean clones are related closely and are currently considered as a single clone (Cordobes/Chilean).[6] In different countries the Cordobes/Chilean clone has replaced quickly the previous predominant clones as in Colombia where it replaced the Pediatric clone[7,9] and in Chile and Argentina where it replaced the Brazilian clone.[4,6] Knowledge about MRSA rates and the molecular typing of *S. aureus* from Peru is very limited. One study showed that all 172 MRSA isolates from three Peruvian hospitals isolated during 2006–2008 had genotypic characteristics of hospital-associated (HA-) MRSA isolates, contrasting the results from Ecuador, Colombia and Venezuela where 74%, 31% and 14% had community-associated (CA)-MRSA molecular characteristics.[10] We recently described the first cases of CA-MRSA soft tissue infections in Peru caused by isolates carrying the Staphylococcal Chromosomal Cassette *mec* (SCC*mec*) IV and the Panton-Valentine leukocidin (PVL) genes.[11]

The present study aimed to describe the antimicrobial resistance patterns and molecular typing of MRSA and MSSA isolates from Peruvian hospitals.

Materials and methods

From April 2008 until June 2009, non-duplicate consecutive *S. aureus* isolates were collected from blood cultures sampled drawn as part of routine patient care at seven general public hospitals in Lima and Callao, two contiguous cities at the coast of Peru. Peru is a middle income country with 30 million inhabitants, of which almost one third is living in Lima and Callao. For logistic reasons no other hospitals from the country were involved. The seven participating hospitals (Table 1) with more than 5000 beds represent approximately 30% of the total hospital bed capacity of Lima and Callao. Patients' age and information about hospital ward were registered. The study was approved by the Ethical Institutional Committee of Universidad Peruana Cayetano Heredia and by the Ethical Committee of Antwerp University.

The participating laboratories stored *S. aureus* isolates on Trypticase Soya Agar tubes (Oxoid LTD, Hampshire, England). Isolates were collected weekly by a collaborator of the Institute of Tropical Medicine Alexander von Humboldt (Lima, Peru), where phenotypic analysis was performed. Isolates were retrieved on mannitol salt agar (Oxoid LTD, Hampshire, England) and mannitol positive colonies were identified as *S. aureus* by Gram stain and positive reactions for catalase, DNA-se and tube coagulase tests. Oxacillin resistance screening was performed as recommended by CLSI.[12] Molecular analysis of the isolates was performed at the department of Medical Microbiology of Maastricht University Medical Center (Maastricht, The Netherlands). Confirmation of methicillin resistance was performed by detection of *mec*A gene using a real-time PCR.[13] The genetic background of all *S. aureus* isolates was determined using *spa* typing, which were clustered into *spa* clonal complexes (*spa*-CC) with the RidomStaphType Type version 1.5 software package(http://www.ridom.de).[13–15] *Spa* typing yields results that are in concordance with typing results obtained by Multi Locus Sequence Typing (MLST).[14,16] SCC*mec* typing was performed with real-time PCR as previously described.[15,17,18] For SCC*mec* typing the following control strains were used: COL, BK2464, ANS46, MW2, and WIS.[18] Clonal lineages as defined by multilocus sequence typing and eBURST analysis were inferred from previous *spa*-MLST mapping.

Antimicrobial susceptibilities were assessed by disk diffusion for penicillin, chloramphenicol, clindamycin, erythromycin, gentamicin, ciprofloxacin, linezolid, rifampicin, and trimethoprim-sulfamethoxazole (TMP-SMX); inducible clindamycin resistance was determined by the D-test disk diffusion method.[12] *S. aureus* ATCC 29213 was used for quality control.

Statistical analyses were performed using the software STATA 10.1 (Statacorp, Texas, USA). Categorical variables were assessed for significance by the chi square test. A p-value <0.05 was considered statistically significant.

Results

A total of 338 *S. aureus* isolates were collected. *S. aureus* accounted for 23.5% of clinically significant organisms recovered from blood cultures in the participating hospitals during the study period, thereby ranking first in all but one hospital, before *Escherichia coli* (17.7%) and *Klebsiella* spp. (17.4%). The MRSA prevalence was 50.0% ($n = 169$) for all hospitals combined, ranging from 44.9% to 60.0% in six hospitals, in the remaining one it was 24.0% (Table 1). The MRSA prevalence was higher among adults as compared to children and neonates (56.6% versus 19.0%, $p < 0.001$) and higher in ICU wards compared to other wards (72.5% and 45.0%, $p < 0.001$) (Table 2).

MSSA isolates comprised 56 *spa* types, 47 of them were clustered into eight associated MLST CC (Table 3). The main MLST CC was CC8, comprising 27.2% of MSSA isolates. The next two most common MLST CC were CC1 (18.9%), and CC15 (13.6%). MRSA isolates were clustered into four associated MLST CC: the majority of them (138/168, 81.7%) were clustered into MLST CC5, with more than two-thirds (95/138, 68.8%) belonging to *spa* type t149. MLST CC5 included 12 other *spa* types: of them, *spa*-types t4088 and t143 comprised 14 and 13 isolates respectively. The other two MLST CC30 and CC8 represented one *spa* type each, i.e. t037 and t148 with 15 and 14 isolates respectively.

About three-quarters ($n = 127$, 75.1%) of MRSA isolates carried SCC*mec* I and comprised uniquely MLST CC5 isolates. SCC*mec* II were detected in nine isolates, eight of them belonged to MLST CC5 (these isolates were distributed among the following *spa* types: t002, t242, t586 and t7130).