BRIEF REPORT

FREQUENCY OF COMMUNITY-ACQUIRED METHICILIN-RESISTANT *Staphylococcus aureus* IN A TERTIARY CARE HOSPITAL IN PERU

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ABSTRACT

In order to determine the frequency of community-acquired methicillin-resistant Staphylococcus aureus (CA-MRSA) isolates and to describe the antimicrobial resistance pattern and genotype, a cross-sectional study was conducted in 2017 at the Hospital Nacional Cayetano Heredia in Lima, Peru. We found a MRSA prevalence of 46.1% in the 115 analyzed S. aureus isolates; most were reported from different secretions (26.4%) and blood (18.9%). We found high co-resistance (>75%) to clindamycin, erythromycin, gentamicin and ciprofloxacin. Regarding SSCmec typification, most of the isolates were identified as hospital-acquired MRSA (HA-MRSA) and a minority of them as CA-MRSA (2.6%). Despite its low prevalence when compared to other Latin American countries (27%), epidemiological surveillance is recommended to control local CA-MRSA dissemination.

Keywords: *Staphylococcus aureus*; Methicillin-resistant *Staphylococcus aureus*; prevalence; epidemiological surveillance; Peru (source: MeSH NLM).

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) was initially described as a bacterium associated with nosocomial infections in patients with prolonged hospital stay, recent surgery, dialysis requirement or presence of invasive medical devices [1-3]. Hospital-acquired MRSA (HA-MRSA) is characterized by being resistant to several families of antimicrobials and carrying the *mecA* gene, contained in the staphylococcal chromosomal cassette *mec* (SCC*mec*) type I, II and III [3-5]. However, in the 1990s, the first cases of community-acquired MRSA infections (CA-MRSA) began to be described in the USA in people without nosocomial risk factors; subsequently infections spread to all continents [4]. The most prevalent CA-MRSA clone is USA300, which characteristically carries SCC*mec* type IV and is usually resistant only to β-lactams [5-6].

In Peru, some imported cases of CA-MRSA were described between 2010-2011 [7]. A study conducted during 2011-2014, using blood cultures, showed that the most prevalent clone in northern South America was the Latin American variant of USA300 (USA300-LV), described in 79%, 72% and 50% of MRSA infections in Colombia, Ecuador and Venezuela, respectively; although no cases were found in Peru [8].

The aim of the study was to determine the frequency of CA-MRSA in *S. aureus* isolates from patients in a hospital in Lima, Peru, and to describe their molecular and antimicrobial resistance characteristics.

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THE STUDY

Design and population

A cross-sectional and descriptive study was conducted during 2017 in patients from the Hospital Cayetano Heredia (HCH) in Lima, Peru. This is a complexity level III-1 public hospital, which has outpatient consultation by specialties and 367 hospital beds. During this period, all *S. aureus* isolates reported in blood, fluid or body secretion by the hospital's microbiology laboratory from pediatric or adult inpatients and outpatients were collected.

Microbiological and molecular analysis

The isolates were transferred to the Tropical Medicine Institute "Alexander von Humboldt" for identification, according to conventional diagnostic procedures. Antimicrobial susceptibility was performed through the Kirby Bauer method and the following antimicrobials were used: cefoxitin, ceftaroline, gentamicin, erythromycin, tetracycline, ciprofloxacin, clindamycin, trimethoprim-sulfamethoxazole, chloramphenicol, rifampicin and linezolid, considering the standard cut-off points [9]. *S. aureus* strain ATCC 29213 was used as quality control.

For molecular analysis, DNA was extracted according to the methodology described by Bouillaut *et al* [10]. The detection of methicillin resistance by identification of the *mecA* gene and subsequent typification of SCC*mec* (types I, II, III, IVa, IVb, IVc and V) were carried out by multiplex PCR, following the methodology described by Zhang *et al* [11]. Likewise, the *luk*F.PV and *luk*S-PV genes, which encode Panton-Valentine leukocidin (PVL), were detected by the PCR method, following the procedures described by Lina *et al* [12].

Those isolates that had discordance between the cefoxitin resistance pattern and the presence of *mecA*, or whose SCC*mec* could not be identified were sent to the Henry Ford Hospital Infectious Disease Research Laboratory (Detroit, Michigan) for typification.

MRSA was defined according to the detection of the *mecA* gene. Because a clinical definition was not used, we defined whether the MRSA isolate was community-acquired based on the type of SCC*mec* and the presence of genes encoding PVL: if isolates carried SCC*mec* type IV or V and had the *luk*F.PV and *luk*S-PV genes, they were considered CA-MRSA. Those carrying SCC*mec* type I, II and III, independent of the presence of *luk*F.PV and *luk*S-PV genes, were considered HA-MRSA [13].

KEY MESSAGES

Motivation for the study: Community methicillin-resistant *Staphylococcus aureus* causes infections with poor response to antibiotics, mainly skin and soft tissue infections. Information in Peru on its presence in hospitals is insufficient, so we sought to determine its frequency in order to establish the necessary preventive biosecurity measures and its resistance profile to identify antibiotics for empirical use.

Main findings: The study confirms the presence of said bacterium in our setting.

Implications: It is necessary to maintain epidemiological surveillance measures to prevent its spread.

Statistical analysis

We used Windows XP Excel 2007 to collect information on the origin of the reported *S. aureus* strains. STATA SE 16 was used for statistical analysis of the data. A descriptive analysis with frequencies and percentages was conducted.

Ethical Considerations

Samples and patient data were processed and stored under strict confidentiality. Each *S. aureus* isolate was assigned a code, and the database was stored with a password, to which only the principal researchers had access. The HCH Institutional Ethics Committee approved the study in 2016, with code 021-017.

FINDINGS

During 2017, 152 *S. aureus* isolates were reported (only one per patient), of which 120 were analyzed in this study. Of these, five isolates were excluded: four had discordant findings between cefoxitin susceptibility and the presence of the *mecA* gene, and one was *S. haemolyticus*.

Of the 115 isolates positive for *S. aureus*, most were obtained from unspecified secretions (21.7%), followed by blood (20.0%), tracheobronchial secretions (14.8%) and skin (14.8%) (Table 1). Of the isolates, 46.1% were identified as MRSA. Among the MRSA isolates (n = 53), the distribution of SCC*mec* types was as follows: I (79.2%), III (1.9%), and IV (7.5%); no isolates with SCC*mec* type II and V were found. In addition, in six isolates (11.3%) the SCC*mec* type could not be determined (Table 2).

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Regarding the antibiotic susceptibility profile, among methicillin-sensitive *S. aureus* isolates (MSSA, n = 62), the highest frequency of resistance was found for erythromycin (22.2%), gentamicin (17.2%) and clindamycin (11.1%). On the other hand, the majority of isolates (>75%) of MRSA showed resistance to clindamycin, erythromycin, gentamicin and, in addition, ciprofloxacin; this co-resistance was more common in isolates carrying SCC*mec* type I and III (n = 43) (Table 2).

Genes encoding PVL were identified in ten isolates (8.7%): six MSSA isolates (9.7%) and four MRSA isolates (7.5%). Of the latter, three isolates were categorized as CA-MRSA, because they carried SCC*mec* type IV; while only 1 corresponded to HA-MRSA, because it carried SCC*mec* type I (Table 3).

DISCUSSION

We analyzed 115 strains (75.7%) of the 152 isolates reported during 2017. Within these, a high frequency of MRSA isolates was found (46.1%) and the frequency of CA-MRSA was 2.6%.

Previous multicenter studies that have evaluated *S. aureus* strains according to their antimicrobial susceptibility and genotype have shown a high prevalence (>40%) of MRSA in Latin America, with a heterogeneous distribution among countries [14,15]. Brazil and Venezuela report the highest frequencies in the region, with 62% and 57%, respectively [15]. In the case of Peru, a frequency of 50%-54% has been reported [14,15]. The frequencies described are similar to those found in our study; however, the comparison with these studies [14, 15] is limited, since only cases of bacteremia were considered. To date, no studies have been conducted in the region that consider all types of isolates.

There are few local studies that evaluate the distribution of MRSA strains and their molecular typification. One research conducted at the same hospital as our study, that considered all *S. aureus* isolates, described a 68% frequency of MRSA in 2002 [16]. Subsequently, a study that included *S. aureus* isolates from all sources in three referral hospitals in Lima [17] showed an overall frequency of 58%, obtaining the molecular characteristics of HA-MRSA in almost all isolates. This shows that the presence of MRSA continues to be prevalent in Peruvian hospitals, and measures should be implemented to contain its dissemination [15].

From the molecular point of view, the SCC*mec* type of MRSA has a varied distribution in Latin America [18,20]. In the northern countries of South America, such as Colombia and Ecuador, the USA300 clone carrying SCC*mec* type IV is the most frequent, followed by the Chilean-Cordovan clone, carrying SCC*mec* type I; while in countries such as Peru and Chile it has been reported that the majority (>90%) of *S. aureus* isolates correspond to the Chilean-Cordovan clone [15].

In Peru, a multicenter study conducted in Lima [17] revealed that MRSA carrying SCC*mec* type I were found with a frequency of 75.2%; furthermore, these isolates showed resistance to ciprofloxacin, clindamycin, erythromycin and gentamicin, similar to what was found in our study. Our findings are similar to previous studies in hospitals in Lima, which confirms that MRSA carrying SCC*mec* type I non-PVL-producing is the most common nosocomial clone in our setting [17,18]. Regarding the emergence of CA-MRSA in the last decade in Peru, several multicenter studies have shown that this is a very infrequent event in Peruvian hospitals [8,14]. In this study, 2.6% of the isolates were found to have molecular characteristics of CA-MRSA, in contrast to neighboring countries such as Brazil, Ecuador, Venezuela and Colombia, where a prevalence of around 27% has been reported [15,20]. This situation requires continuous surveillance because if this proportion increases significantly, there would be an impact on the empirical antibiotic treatment for skin and soft tissue infections, its most frequent presentation.

Likewise, this study found a frequency of genes encoding PVL of 8.7%, with greater distribution in the MSSA group. This exotoxin was first described in 1932 in sensitive strains, as a factor associated with severe skin infections and necrotizing pneumonia [19]. Subsequently, it was described

Table 1. Source of *Staphylococcus aureus* isolates.

Sample type	Total	MSSA	MRSA
	N =115 n (%)	N =62 n (%)	N =53 n (%)
Unspecified secretion	25 (21.7)	11 (17.7)	14 (26.4)
Blood	23 (20.0)	13 (21.0)	10 (18.9)
Bronchial secretion	17 (14.8)	6 (9.7)	11 (20.8)
Skin	17 (14.8)	13 (21.0)	4 (7.5)
Articular bone	3 (2.6)	1 (1.6)	2 (3.8)
Umbilical	1 (0.9)	1 (1.6)	0 (0.0)
Vaginal	2 (1.7)	2 (3.2)	0 (0.0)
Fistula	1 (0.9)	0 (0.0)	1 (1.9)
Urine	1 (0.9)	1 (1.6)	0 (0.0)
Other	3 (2.6)	1 (1.6)	2 (3.8)
Unknown	22 (19.1)	13 (21.0)	9 (17.0)

MSSA: methicillin-sensitive *S. aureus;* MRSA: methicillin-resistant *S. aureus.*

Table 2. Antimicrobial resistance of *Staphylococcus aureus* according to the presence of the *mecA* gene chromosomal cassette type (n = 115).

Drugs	MSSA (n = 62)	MRSA (n = 53)			
	n (%)	n (%)			
		Total	SCC*mec* type I y III [a]	SCC *mec* type IV	Not typifiable
Total	62 (53.9)	53 (46.1)	43 (81.1)	4 (7.5)	6 (11.3)
Erythromycin	14 (22.2)	49 (92.5)	42 (97.7)	2 (50)	5 (83.3)
Gentamicin	11 (17.7)	41 (77.5)	38 (71.7)	0 (0.0)	3 (50)
Clindamycin	7 (11.1)	49 (92.5)	43 (100)	0 (0.0)	3 (50)
Tetracycline	6 (9.7)	1 (1.9)	1 (2.3)	0 (0.0)	0 (0.0)
Ciprofloxacin	4 (6.4)	45 (84.9)	41 (95.3)	0 (0.0)	4 (66.7)
Trimethoprim-sulfamethoxazole	4 (6.5)	1 (1.9)	1 (2.3)	0 (0.0)	0 (0.0)
Rifampicin	3 (4.8)	4 (7.5)	3 (5.7)	0 (0.0)	1 (16.7)
Linezolid	1 (1.6)	2 (3.8)	2 (4.7)	0 (0.0)	0 (0.0)
Chloramphenicol	0 (0.0)	1 (1.9)	1 (2.3)	0 (0.0)	0 (0.0)
Ceftaroline	0 (0.0)	3 (5.7)	3 (7.0)	0 (0.0)	0 (0.0)

MSSA: methicillin-sensitive *S. aureus*; MRSA: methicillin-resistant *S. aureus*; SCC*mec*: staphylococcal chromosomal cassette *mec*.
[a] Only one isolate carrying SCC*mec* III was found.

that its production could be considered a marker for identification of resistant strains, especially CA-MRSA [19]. However, this theory is currently controversial, since the genes encoding PVL are reported in HA-MRSA and MSSA strains. A study conducted in three hospitals in Lima [17] described a low production of PVL (9.1% of the isolates analyzed), with a higher distribution in the MSSA group than in the MRSA group, which is similar to what was found in our study. These findings favor the current theory that the presence of PVL is not a reliable marker for the identification of CA-MRSA strains.

Table 3. Panton-Valentine leukocidin identified in *S. aureus* isolates.

Type of isolate (n =115)	Negative PVL	Positive PVL	Total
	n (%)	n (%)	
S. aureus	105 (91.3)	10 (8.7)	115
MSSA	56 (90.3)	6 (9.7)	62
MRSA	49 (92.5)	4 (7.5)	53
SSC*mec* type			
I	41 (97.6)	1 (2.4)	42
III	1 (100)	0 (0.0)	1
IV	1 (25.0)	3 (75.0)	4
NT	6 (100)	0 (0.0)	6

NT: not typifiable; MSSA: methicillin-sensitive *S. aureus*; MRSA: methicillin-resistant *S. aureus*; SCC*mec*: staphylococcal chromosomal cassette *mec*; PVL: Panton-Valentine leukocidin.

The first limitation of this study was that the number of isolates analyzed was small, which could have altered the true prevalence of CA-MRSA isolates. This was due to the limited availability of reported *S. aureus* isolates during the study period and, probably, to the fact that this institution does not perform systematic culture sampling in cases with suspected skin and soft tissue infection, the most frequent presentation of this infection, or that it is performed after the initiation of antibiotics. The second limitation of the study was that the complete review of medical records was not included, which prevented us from completing the clinical definition of HA-MRSA and CA-MRSA. In addition, this study did not allow us to evaluate whether the samples obtained corresponded to cases of infection or colonization, which would provide more information on the impact of the presence of this bacterium in our setting. In addition, there were 11.3% of MRSA isolates in which the SSC*mec* type could not be identified. The third limitation is related to the external validity of the study. The study was conducted in a public referral hospital located in the northern area of the city of Lima, so the information described only corresponds to that population. This, together with the small number of samples analyzed, limits the extrapolation of the results. However, the intention of our study is to draw attention to the importance of epidemiological surveillance of multidrug-resistant bacteria.

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Our study shows a low frequency of CA-MRSA in Lima. However, we consider that close epidemiological surveillance should be continued and studies should be expanded, even more so in the context of increasing migration from countries with higher prevalence.

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