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ORIGINAL ARTICLE

Dynamics of nasal carriage of methicillin-resistant *Staphylococcus aureus* among healthcare workers in a tertiary-care hospital in Peru

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Abstract The study aims were to describe the frequency and dynamics of methicillin-resistant *Staphylococcus aureus* (MRSA) carriage among healthcare workers (HCWs), and to compare the molecular epidemiology of MRSA isolates from HCWs with those from patients with bacteremia. HCWs were interviewed and three nasal swabs were collected in a hospital in Lima, Peru, during 2009–2010. Consecutive *S. aureus* blood culture isolates from patients with bacteremia in the same hospital were also collected. SCC*mec*, multilocus sequence typing (MLST), and *spa* typing were performed. Persistent carriage was defined if having at least two consecutive cultures grown with *S. aureus* harboring an identical *spa* type. Among 172 HCWs included, the proportions of *S. aureus* and MRSA nasal carriage during first sampling were 22.7 % and 8.7 %, respectively. From 160 HCWs who were sampled three times, 12.5 % (20/160) were persistent *S. aureus* carriers and 26.9 % (43/160) were intermittent carriers. MRSA carriage among persistent and intermittent *S. aureus* carriers was 45.0 % (9/20) and 37.2 % (16/43), respectively. Fifty-six *S. aureus* blood culture isolates were analyzed, and 50 % (n=28) were MRSA. Multidrug resistant ST5-*spa* t149-SCC*mec* I and ST72-*spa* t148-SCC*mec* non-typeable were the two most frequent genotypes detected among HCWs (91.7 %, i.e., 22/24 HCW in whom MRSA was isolated in at least one sample) and patients (24/28, 85.7 %). In conclusion, we found high proportions of MRSA among persistent and intermittent *S. aureus* nasal carriers among HCWs in a hospital in Lima. They belonged to similar genetic lineages as those recovered from patients with bacteremia.

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Introduction

Nasal carriage of methicillin-resistant *Staphylococcus aureus* (MRSA) among healthcare workers (HCWs) plays a role in its transmission in the hospital [1]. Different patterns of carriers have been described, comprising persistent, intermittent and non-carriers [2]. MRSA carriage rate in HCWs is estimated to be 4.6 %, but few studies have assessed the pattern of MRSA carriage through longitudinal studies [3]. Our aims were to determine the frequency of MRSA nasal carriage among HCWs in a hospital in Peru, to describe the dynamics of carriage (intermittent versus persistent) and to compare the molecular characteristics of *S. aureus* collected from both HCWs and patients with bacteremia.

Methods

We conducted a longitudinal study (December 2009–2010) at Hospital Nacional Cayetano Heredia (HNCH), a 420-bed tertiary care-level hospital of Lima, Peru. At the time of the

study, HNCH did not have a MRSA infection control policy (no contact isolation, no MRSA screening).

Physicians, nurses and nursing assistants were assessed for *S. aureus* nasal carriage by three consecutive samplings over a one-year period (intervals of 2–8 months). Demographic data included age, gender, occupation, ward (Medicine, Pediatrics, Surgery or Obstetrics/Gynecology [Ob&Gyn]), duration of employment, hospital admission during the previous year, co-morbidities and antimicrobial use during the last month [1]. Among each subgroup, participants were selected by convenience at the moment of sampling. Nasal swabs were taken at any time during the working day. One sample obtained by inserting a cotton-tipped swab into both anterior nares was transported, and inoculated onto Mannitol Salt Agar (Oxoid LTD, Hampshire, England) and incubated at 37 °C for 24 h. One mannitol-positive colony per plate was analyzed.

All non-duplicate consecutive *S. aureus* isolates recovered from blood cultures sampled as part of routine patient care in HNCH during 2009–2010 were collected. Healthcare-associated infection was defined if the patient (i) had been hospitalized for more than 2 days at the time of the blood cultures, (ii) was under dialysis support, or (iii) declared a history of hospitalization during the previous month.

S. aureus were identified by Gram stain and positive reactions for catalase, DNA-se (Oxoid, Hampshire, England) and tube coagulase tests (Remel, Kent, England). Antimicrobial susceptibility for penicillin, chloramphenicol, clindamycin, erythromycin, gentamicin, ciprofloxacin, rifampicin, and trimethoprim-sulfamethoxazole (TMP-SMX) was assessed by disk diffusion using an inoculum equivalent to 0.5 McFarland standard [4]. *S. aureus* ATCC 25923 and 43300 were used for quality control. Detection of 16S rRNA, *mec*A and *nuc* genes and *spa* typing were performed in all isolates [5]. MRSA isolates were further analyzed for detection of Panton-Valentine Leukocidin (PVL)-encoding genes *luk*S-*luk*F by PCR, SCC*mec* types by multiplex PCR for the *mec* and *ccr* gene complexes [5–7]. Multilocus sequence typing was performed on one randomly selected MRSA isolate of each *spa* type [8].

Persistent carriage was defined as having at least two consecutive cultures grown with *S. aureus* harboring an identical *spa* type. Intermittent carriers were referred to all other combinations of positive *S. aureus* culture results and *spa* types [9].

Clinical and demographic variables were assessed for association with MRSA colonization (using the first sampling). Chi-square test was used to compare categorical and dichotomous variables. Median values were compared using the Wilcoxon rank-sum test. Stata 10.0 software (Statacorp, Texas, USA) was used and the level of significance was set at 0.05.

Results

One hundred seventy-two HCWs (from a total of 1038) were recruited and 493 samples were obtained. The participants' median age was 36.5 years (range 22–65), and 72.6 % were females. Based on the first sampling, the frequency of *S. aureus* and MRSA nasal carriage was 22.7 % and 8.7 %, respectively (Table 1); the frequency of MRSA carriage was significantly lower (2.1 %) in Ob&Gyn and Pediatrics combined compared to Medicine and Surgery combined (13.3 %) (p=0.015) (Table 1); it was also higher among nurse assistants (12.5 %) compared to physicians (6.0 %) and nurses (8.2 %) but this difference was not statistically significant (p=0.22). The frequency of *S. aureus* and MRSA carriage was 22.8 % and 7.6 % in the second sampling (n=171) and 22.4 % and 7.5 % in the third sampling (n=161). No significant factor associated with MRSA compared to methicillin-susceptible *S. aureus* (MSSA) carriage was found.

By molecular typing, 34 of 36 MRSA isolates were grouped into two clones as defined by the combination of MLST, *spa* type and SCC*mec* type: ST172-*spa* t148-SCC*mec* non-typeable (NT) (n=24) and ST5-*spa* t149-SCC*mec* I (n=10) (Table 2). These two genotypes were co-resistant to erythromycin, clindamycin and ciprofloxacin. No isolate carried the PVL-encoding genes. The 36 MRSA isolates were recovered from 24 HCWs and (22/24) (91.7 %) carried either ST172-*spa* t148-SCC*mec* NT (n=15) and -ST5-*spa* t149-SCC*mec* I strains (n=7);

Table 1 *Staphylococcus aureus* and methicillin-resistant *S. aureus* (MRSA) nasal carriage among healthcare workers (HCWs) based on ward and position. Data as obtained during first sampling

Variable	Number	Carrier	
		% of HCWs	
		S.aureus	MRSA
Ward			
Medicine	50	22.0	12.0[a]
Surgery	48	22.9	14.6[a]
Pediatrics	49	30.6	4.1[a]
OB&Gyn	25	8.0	0[a]
Position			
Physician	67	25.4	6.0[b]
Nurse	49	20.4	8.2[b]
Nurse assistant	56	21.4	12.5[b]
All	172	22.7	8.7[b]

OB&Gyn Obstetrics and Gynecology

[a] p=0.015 Medicine and Surgery versus Pediatrics and Ob&Gyn

[b] p=0.25 Nurse assistant versus physicians and nurses

Table 2 Phenotypic and molecular characteristics of methicillin-resistant *Staphylococcus aureus* isolates from healthcare workers and patients with bacteremia

MLST	*spa* type	Number of isolates	Number of subjects	SCC*mec* type	Antibiotic resistance
From healthcare workers					
ST72	t148	24	15	NT	PEN, OXA, ERY, CLI, CIP
ST5	t149	10	7	I	PEN, OXA, ERY, CLI, CIP, GEN
ND	t002	1	1	II	PEN, OXA, ERY, CLI, CIP, GEN
ND	t288	1	1	I	PEN, OXA, ERY, CLI, CIP, GEN
Total		36	24		
From patients with bacteremia					
ST5	t149	18	18	I	PEN, OXA, ERY, CLI, CIP, GEN
ST72	t148	6	6	NT	PEN, OXA, ERY, CLI, CIP
ND	t037	4	4	III	PEN, OXA, ERY, CLI, CIP, GEN
Total		28	28		

MLST mutlilocus sequence type, *ND* not determined, *NT* non-typeable, *SCCmec* element with *ccr* type 2, no *mec* gene complex detected, *PEN* penicillin, *OXA* oxacillin, *ERY* erythromycin, *CLI* clindamycin, *CIP* ciprofloxacin, *GEN* gentamicin

they were distributed over the four wards except for the first one which was not present in HCWs working in Ob&Gyn.

Among 160 HCWs who were sampled three times, 43 (26.9 %) were intermittent carriers, among them 16 (37.2 %) carried MRSA. Twenty (12.5 %) HCWs were persistent carriers; nine of them (45.0 %) carried MRSA (Fig. 1).

During the study period, 56 non-duplicate consecutive *S. aureus* isolates were collected from blood cultures, 28 (50 %) of them were MRSA. All MRSA cases were healthcare-associated infections. Among them, ST5-*spa* t149-SCC*mec* I (n=18, 64.3 %) and ST172-*spa* t148-SCC*mec* NT (n=6, 21.4 %) were the most frequent genotypes detected; antimicrobial resistance patterns were identical to those described among HCWs (Table 2). No MRSA isolate carried the PVL genes.

Discussion

This study showed a high frequency of MRSA nasal carriage among HCWs in a Peruvian hospital. Among the persistent nasal *S. aureus* carriers, 45 % of them carried MRSA isolates. The predominance of two genotypes ST172-*spa* t148-SCC*mec* NT and ST5-*spa* t149-SCC*mec* I found in HCWs and patients with bacteremia is highly suggestive of an active transmission between the two groups.

The frequency of MRSA nasal carriage among HCWs was higher compared to the 4.6 % worldwide estimate that was based on a review of 104 studies. However, it is important to consider that most of these studies were performed in high income countries [3]. Literature from Latin America is sparse. After searching English, Spanish and Portuguese publications displayed in PubMed, Lilacs and Scielo databases in the last

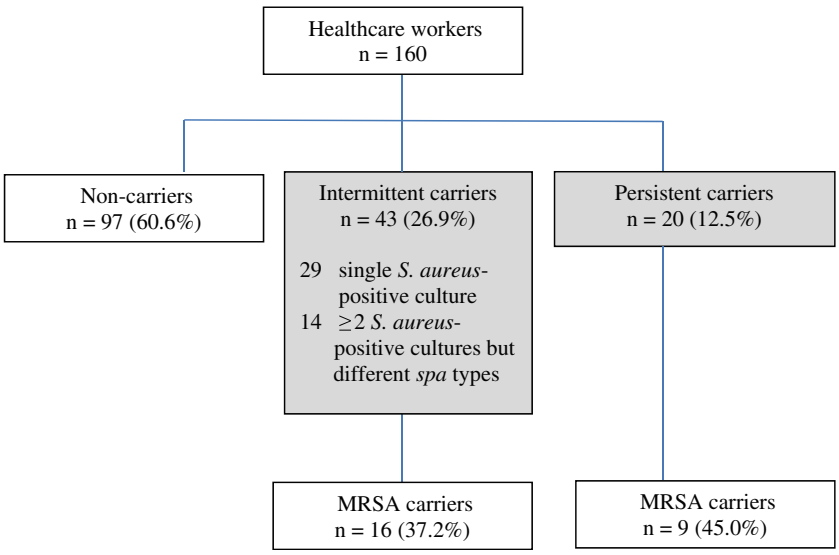

Fig. 1 *Staphylococcus aureus* carriage pattern among healthcare workers

20 years, we only found 13 studies from seven (of 17) Latin American countries which assessed MRSA carriage rates among HCWs (Supplementary Table); rates varied between 0 % and 39.5 % [10–22]. As expected, sample size and microbiology methods varied and most of them included other than nasal samples [10, 11, 13, 14, 19–22]. Two of these studies were done in Peru, one did not find any case of MRSA carrier that may be explained by the fact that this study took place in an out-patient setting [19]. The other study described 20 % of MRSA colonization among 45 workers of a hospital; a total of 26 MRSA strains were isolated but most of them were obtained from throat swabs [20]. This latter event may explain why they found a higher rate of MRSA carriage compared with us since we only included nasal carriage.

The high frequency of MRSA carriage among HCWs in our study may be related to different factors such as lack of implementation of patient isolation (i.e., rooms are shared by two to six patients), absence of active screening for MRSA in patients and HCWs, and low adherence to hand hygiene procedures. We did not find a statistical difference in the MRSA rates based on occupation; however, the MRSA rates among nurse assistants and nurses were slightly higher compared to those among physicians. Other studies have shown the same trend [23]. This may be explained by the high number of patients assigned to the nurse/nurse assistant staff in the inpatient wards.

Our findings further indicated that the frequency of MRSA carriage was slightly higher among persistent compared to intermittent carriers. Data about predominance of MRSA among persistent versus intermittent carriers are variable and limited [24, 25]. Hospitals from high income countries include MRSA decolonization of HCWs as part of their infection control policies; as a result, there are few longitudinal studies showing the natural history of colonization with MRSA. Regarding the frequency of MRSA in *S. aureus* bacteremia, a high rate of MRSA has been described previously in a multicenter study in Peru with an overall rate of 50 % [26] and in a multicenter study involving four Latin American countries (Colombia, Ecuador, Peru and Venezuela), with an overall rate of 39 % [27].

There were only two predominant genotypes among MRSA isolates in the present study, ST172-*spa* t148-SCC*mec* NT and ST5-*spa* t149-SCC*mec* I. MRSA *spa* t148, which was isolated the most frequently from HCWs, has been previously recovered in 8.3 % of invasive MRSA isolates from Peruvian hospitals [26]. This genotype which has been rarely seen in European countries is a major community-associated MRSA clone in Asia [28, 29]. The multidrug resistant ST5-*spa* t149-SCC*mec* I genotype corresponds to the predominant hospital-acquired MRSA clone in Peru and other countries of South America such as Paraguay, Argentina, Chile, Ecuador, Colombia and Venezuela [26, 27, 30].

Among the limitations of this study, it should be noted that only nasal swabs were collected and no selective enrichment cultures for MRSA were used which may have caused an underestimation of *S. aureus* and MRSA frequency and that swab sampling was taken at any time of the day. Further, a bias in the selection of participants cannot be excluded. Seasonality variations among carriers were not assessed in this study. One of the strengths of this study was that the sample size was larger than many studies in the literature. Additionally, three consecutive samples were taken in most of the participants.

Conclusions

We found a high frequency of MRSA colonization in HCWs. Since this was a longitudinal study we were able to define the frequency of persistence and intermittent *S. aureus* carriers showing a high MRSA proportion among both of them. We also showed two similar *S. aureus* genotypes circulating among patients and HCWs. These results reveal the need to implement infection control policies in order to reduce the transmission of multidrug resistant microorganisms among HCWs and patients.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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