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ORIGINAL RESEARCH

Methicillin-Resistant *Staphylococcus aureus* Nasal Colonization Among Health Care Workers of a Tertiary Hospital in Ecuador and Associated Risk Factors

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Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) is resistant to most of the commonly used antibiotics and is therefore a public health issue. Colonization with MRSA is a risk factor for infection or transmission.

Purpose: To determine the prevalence of colonization with *Staphylococcus aureus* (SA) and MRSA strains in health care workers (HCWs) at a tertiary hospital in Ecuador and to determine the risk factors associated with carriage.

Methods: Out of a cohort of 3800 HCWs, 481 individuals from different hospital departments were randomly selected, and a single nasal swab was collected. Detection of SA and MRSA was carried out with the LightCycler® MRSA Advanced Test. A questionnaire was performed that gathered demographic and occupational information of the participants to determine risk factors for MRSA colonization. Statistical analysis was performed with univariate and multivariate analysis and the R-software version 4.0.2.

Results: Colonization with SA and MRSA occurred in respectively 23.7% (95% CI, 22.7–24.6) and 5% (95% CI, 3.39–7.58) of the individuals. The multivariate analysis showed that being older in age (OD 1.09) and being male (OD 2.78) were risk factors for SA and MRSA colonization (p-value < 0.001). Previous use of antibiotics or the use of nasal ointments diminished the colonization rates of SA (24% versus 3.7% and 10.1% respectively).

Conclusion: About 20% of the HCWs who were colonized with SA were colonized with MRSA, representing a risk for nosocomial infections and hospital outbreaks. Active monitoring and a decolonization treatment of the HCWs can reduce these risks.

Keywords: *Staphylococcus aureus*, SA, antibiotic resistance, methicillin-resistant *Staphylococcus aureus*, MRSA, health care workers, HCWs, colonization, risk factors

Introduction

Bacterial infections caused by resistant pathogens continue to be a growing threat to public health.[1,2] Currently, it has been estimated that more than 70% of the bacteria that cause nosocomial infections are resistant to at least one of the drugs commonly used for treatment. Moreover, it has been estimated that by 2050, the problem of bacterial resistance will increase drastically and cause several millions deaths per year.[3]

One of the most important pathogens in humans is *Staphylococcus aureus* (SA), commonly associated with high rates of morbidity and mortality, and one of the

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main causes of organ necrosis, sepsis, endocarditis, and osteomyelitis.[4] SA is notorious for its ability to acquire resistance to a variety of antibiotics. An example is methicillin-resistant *S. aureus* (MRSA), which causes infections that are extremely difficult to treat, especially because drug alternatives are limited.[4–6]

Humans are frequently colonized with SA and at several stages throughout their lives. The body site most often colonized is the anterior nares. Other sites frequently colonized include the axillae, hands, throat, perineum, vagina, and gastrointestinal tract. Colonization with MRSA has been associated with an increased risk of symptomatic and serious infections. In some settings, health care workers (HCWs) exhibit a high prevalence of MRSA colonization and are likely to be important transmitters of MRSA to patients through contact with hands or the airborne spread of SA in association with an upper respiratory tract infection. In this context, it is vital to mention that a high percentage of nosocomial infections in intensive care units in hospitals is due to MRSA infections.[1,7,8] In developing countries, MRSA infections have become an emerging problem, considering the high risk of mortality associated with resistance,[9] especially for patients with prolonged hospital stays.[10]

Worldwide, there are several guidelines and recommendations for the control and prevention of SA infection and colonization in hospital workers and patients.[4,11,12] The control is based on simple hygiene measures and a rigorous "search and destroy" approach,[13] based on screening patients and staff for MRSA colonization, isolating affected patients, asking infected HCWs to not come into work, and employing decolonization treatments with an antibacterial agent for both groups.

Although there are several genes potentially involved in methicillin resistance, the main mechanism described for MRSA isolates from humans, and especially in hospital environments, is associated with the *mecA* gene that belongs to the SSCmec chromosomal cassette.[14,15] MRSA surveillance using molecular assays that detect this cassette increases the sensitivity of detection and can be an important component for decreasing the prevalence of MRSA infection and bacteremia.[16] An example of a molecular tests is the LightCycler® MRSA Advanced Test (Roche Diagnostics, Basel, Switzerland) that has been shown to be more sensitive than culture on the chromogenic medium CHROMagar MRSA.[17] Moreover, the molecular assay provides fast results (within a few hours), speeding up the diagnosis, and it thus may contribute to lowering the rates of both morbidity and mortality. Traditional culture methods need at least an 18–24 hour incubation period before results are available.

In Ecuador, few hospitals carry out active surveillance for MRSA carriers. The present study aims to demonstrate the prevalence of MRSA carriage in hospital workers at a tertiary hospital in Ecuador using the LightCycler® MRSA Advanced Test. This is a commercially available real-time PCR assay for the direct detection of MRSA nasal colonization by targeting the staphylococcal cassette chromosome mec (SCCmec)-orfX junction. This test facilitates detecting all five of the known SCCmec types and distinguishes MRSA from Methicillin-responsive SA.

Materials and Methods

Ethics

The research ethical review Committee of the Hospital de Especialidades Carlos Andrade Marín (HECAM) reviewed and approved the protocol and the informed consent for this study. All patient data were anonymous and subjects' information and privacy were fully protected. The study was conducted according to the Declaration of Helsinki developed by the World Medical Association.

Study Population and Sample

This exploratory and cross-sectional study evaluated a cohort of health care workers (HCWs) at the largest hospital in Quito, the capital of Ecuador. We randomly tested 481 workers from different departments of this hospital between January and December 2015 for SA colonization. The sample size was calculated using R software version R-4.0.2, based on the total number of workers (approximately 3800) at the hospital in 2015, an expected frequency of 2.4% of MRSA carriers, and an error margin of 5%. This frequency of MRSA carriers was based on a previous study by Ruiz et al (2014) into HCWs from 3 hospitals in Quito (12).

A single nasal sample was collected from each person using an individual sterile swab that after sampling, was directly immersed in DNA transport medium (Roche) and then stored at –20°C until further processing.

Molecular Detection of MRSA

SA and MRSA were detected with the LightCycler® MRSA Advanced Test (Roche Molecular Diagnostic, Germany). For lysis and DNA extraction, the swab heads were incubated in the sample preparation buffer

and mechanically lysed using a MagNA Lyser Instrument (Roche Diagnostics, Basel, Switzerland) according to the manufacturer's guidelines. The MagNA Lyser is a benchtop device that automatically disrupts cells or other biological materials. The instrument facilitates the production of a supernatant containing nucleic acids and proteins suitable for subsequent purification, extraction or analysis. The isolated DNA was used in the LightCycler® MRSA Advance Test, a real-time multiplex PCR that detects two targets: the resistance determinant mecA, and the SA-specific gene *sau*. The amplification peaks were analyzed using Micro Analysis Software (MAS) (Roche Molecular Diagnostic, Germany).

Risk Factors Survey

A survey was performed to determine the risk factors for MRSA colonization for all participants. The risk factors were related to the main characteristics of the population and the nature of their work in the hospital. The survey asked for age, sex, work activity, hospital working area, use of personal protective equipment, use of antibiotics prior to nasal swabbing, use of nasal ointments, and training in infection control measures.

Statistical Analysis

Statistical analysis was performed using R Version R-4.0.2. The confidence level was set to 95% and all results with a p-value < 0.05 were considered significant. Univariate analysis was performed with a Pearson chi-squared test for the different variables. For the multivariate analysis, a backward stepwise logistic regression model for variables with $p < 0.25$ was built.

The univariate logistic regression was performed for demographic characteristics and other variables to estimate the odds ratios (OR) for potential risk factors associated with SA colonization. A two-tailed test with a p-value < 0.05 was considered statistically significant. Any variables with $p < 0.05$ in the univariate analysis were carried forward in multivariate logistic regression to analyze the prognostic indicators for SA colonization.

Results

From January to December 2015, 481 HCWs were sampled. The demographic data of the participants in this study can be found in Table 1. Most participants (72%) were women and the average age of the participants was 36.6 years (SD 10.32).

According to their work activities, 39.1% (n=188/481) of the personnel were doctors, 46.5% (223/481) nurses, 3.3% (16/481) were laboratory technicians and 11.2% (54/481) were cleaning and maintenance workers. Regarding health care attention, the physicians were classified into two groups: surgeons (25.5%, n=48/188) and clinicians (74.5%, n=140/188). See Table 1.

SA Colonization

The molecular detection of SA in nasal swabs showed that 23.7% (95% CI, 22.7–24.6) or 114 of the 481 participants were colonized. No significant differences were found in colonization rates for SA between the different age groups. Male workers had a higher colonization rate (38%) (95% CI, 0.28–0.50) than the female workers (19%) (95% CI, 0.15–0.24). SA Colonization was noted among participants of all work activities and health care attention areas, including those HCWs who do not have direct contact with patients. Prevalence was highest among laboratory technicians (31%), followed by doctors (28%), and cleaning and maintenance workers (26%). Nurses had a significant lower colonization rate of 19%. See Table 1. Concerning the molecular detection of MRSA prevalence of 5% (95% CI, 3.39–7.58) was found with 25 of the 481 HCWs colonized with MRSA and mostly affecting the older age group and men.

Risk Factors for MRSA Colonization

Age and sex proved to be important risk factors for MRSA carriage. Regarding the oldest age group of HCWs (52–67 years old) showed a significantly higher prevalence of MRSA of 14% [6.26–25.80] 95% CI (n =8/57), compared to the other age groups. Additionally, the prevalence of MRSA in relation to sex was 7.8% (95% CI, 3.81–13.90) or 10/128 males, and 4.3% (95% CI, 2.45–7.07) or 15/345 females. (See also Table 2). The use of personal protective equipment and participation in a training in infection control measures had no significant impact on SA or MRSA carriage. However the numbers of HCWs dat did not use protective equipment or did not participate in a infection control training were low and these results may or may not be inconclusive. Antibiotics use prior to nasal swabbing (24% versus 3.7%) or the use of nasal ointments (24% versus 10.1%) decreased the colonization rates of MRSA.

Discussion

To our knowledge, this is the first report of SA and MRSA colonization in a tertiary hospital in Ecuador that included

Table 1 *S. aureus* Colonization and MRSA Prevalence Among Health Care Workers, Detected with the LightCycler® MRSA Advanced Test

Demographic Characteristics		n (N=481)	*S. aureus* Colonization		MRSA	
			Carriage Frequency (n)	% Prevalence (95% CI)	Carriage Frequency (n)	% Prevalence (95% CI)
			114	24	25	5
Age (years)	20–35	262	67	26 (0.20–0.32)	5	17 (0.62–4.40)
	36–51	159	32	20 (0.14–0.28)	11	65 (3.50–12.04)
	52–67	57	12	21 (0.11–0.37)	8	14 (6.26–25.80)
	Age not available	3	3	ND	1	ND
Gender	Male	128	48	38 (0.28–0.50)	10	78 (3.81–13.90)
	Female	345	66	19 (0.15–0.24)	15	43 (2.45–7.07)
	Gender not available	8	0	ND	0	ND
Work Activity	Doctor; (48 surgeons and 140 clinicians)	188	52	28 (0.21–0.36)	9	48 (2.21–8.89)
	Nurse	223	43	19 (0.14–0.26)	14	63 (3.47–10.31)
	Lab Technicians	16	5	31 (0.10–0.73)	1	63 (0.16–30.23)
	Cleaning workers	54	14	26 (0.14–0.43)	1	19 (0.47–9.89)
Health Care Attention	Inpatient "Surgical": surgeons (48) + nurses (235) + cleaning workers (5)	288	63	22 (0.17–0.28)	16	56 (3.21–8.87)
	Inpatient "Clinical": clinicians (140) + cleaning workers (6)	146	39	27 (0.19–0.37)	8	55 (2.40–10.51)
	Laboratory	16	5	31 (0.10–0.73)	1	63 (0.16–30.23)
	Others	31	7	23 (0.09–0.47)	0	0
Infection control measures	Previous antibiotic use	143	18	3.7 (0.10–0.15)	5	3.5 (3.47–6.51)
	Use of nasal ointment	110	12	10.1 (0.10–0.15)	3	2.7 (2.67–5.72)
	Use of personal protective equipment (masks and gloves)	429	105	24.5 (0.24–0.28)	23	5.4 (5.38–7.53)
	Hygiene training	399	101	25.3 (0.25–0.29)	22	5.5 (5.48–7.73)

Abbreviations: CI, confidence interval; MRSA, Methicillin-Resistant *Staphylococcus aureus*.

HCWs of all work activities at the hospital. 114 of the 481 (23.7%) workers were carriers of SA. This prevalence is relatively low if we compare it to other similar studies, for instance a report from Brazil in 2014 that found a prevalence of 33% in HCWs.[18] Another study in Quito in 2019 reported an even higher colonization rate of 57.8% (186/322) among medical students.[19] However, it should be mentioned that the 2019 study in Quito involved nasal and throat swabs, whereas our study only analyzed nasal swabs. Some studies have shown that pharyngeal colonization can be higher than nasal colonization.[20] In addition, other parts of the body, such as the skin or digestive tract, can also function as persistent or temporary reservoirs of this pathogen.[21] Moreover, sampling time affects the detection of colonization. In a longitudinal study with nasal swabs taken every 4 weeks, 58% of the HCWs were found to have SA at least once during the study, while the median nasal carriage rate of SA at 4-weekly time points was 36.9%.[22]

The prevalence of MRSA in our study was 5% (95% CI, 3.39–7.58). This finding is in line with previous researchers in Brazil and Ethiopia, who reported 5.1% and 5.8% of MRSA colonization respectively.[18,23] In Ecuador, the prevalence of MRSA, detected in this study, was higher than a previous study carried out in Quito[10] that reported a prevalence of 2.4% but lower than the study into medical students[19] that reported a 7.1% prevalence of MRSA. A similar prevalence was demonstrated in other Latin American countries, such as Argentina, where a study in hospitals showed that the overall MRSA acquisition rate was 2.3/1000 patient-days-at-risk with a MRSA acquisition prevalence of 1.96%[24] Of course, there are studies that have shown a much higher MRSA colonization prevalence. In

Table 2 Univariate and Multivariate Analysis of Risk Factors for Nasal Carriage of Methicillin-Sensitive and Methicillin Resistant *S. aureus* Among HCWs

Variable	Detail of Variable	Univariate Analysis		Multivariate Analysis	
		p	OR (95% CI)	*p*	OR (95% CI)
Age	19.7–59.0 (years)	0.917	0.96 (0.49–1.87)	<.001	1.09(1.04–1.14)
Gender	Female (intercept)				
	Male	<.001	2.38 (1.52–3.72)	0.04	2.78 (1.04–7.39)
Work Activity	Doctor (intercept)				
	Nurse	0.759	1.18 (0.39–3.58)	0.722	0.65 (0.06–6.58)
	Laboratory Technician	0.046	0.62 (0.39–0.99)	0.987	–
	Cleaning workers	0.801	0.91 (0.46–1.82)	0.906	1.06 (0.39–2.86)
Healthcare attention	Inpatient "surgical" (intercept)				
	Inpatient "clinical"	0.038	0.61 (0.39–0.97)	0.651	1.23 (0.49–3.04)
	Laboratory	0.881	1.08 (0.35–3.32)	0.809	1.30 (0.15–11.33)
	Others	0.442	0.69 (0.28–1.74)	0.909	–
Infection control measures	Previous antibiotic use (yes)	<.001	0.36 (0.21–0.63)	0.248	0.52 (0.17–1.55)
	Use of nasal ointment (yes)	0.827	0.92 (0.46–1.83)	0.955	0.96 (0.24–3.7)
	Hygiene training (yes)	0.205	0.61 (0.29–1.30)	0.257	0.42 (0.09–1.85)
	Use of personal protective equipment (masks and gloves) (yes)	0.348	1.54 (0.62–3.82)	0.509	1.74 (0.33–9.08)

Abbreviations: CI, confidence interval; MRSA, Methicillin-Resistant *Staphylococcus aureus*; OR, Odds Ratio.

HCWs in Iran[25] and Portugal,[26] prevalence rates of MRSA carriage of respectively 18% and 17.2% have been reported. In southwest of Iran, in a hospital setting nearly 80% of the *S. aureus* strains isolated from burn patients were MRSA.[27]

The differences in MRSA colonization prevalence are not easy to explain but could be related to differences in local infection control measures, frequency of antibiotic use in the population, sensitivity of the method used for detecting MRSA strains, and the characteristics of the sample population.[28–31]

Identifying risk factors for MRSA carriers remains crucial for control programs that apply a targeted screening approach, for instance a "search and destroy" policy.[32] A key risk factor in our study was sex (being male). The prevalence of MRSA colonization in males was significantly higher than in women (7.8% versus 4.3%, p<0.01) and this has also been described by other researchers.[18,25,33] Additionally, older individuals are more likely to be colonized with MRSA. This coincides with several other investigations in which being older and male are risk factors for colonization, probably due to hygiene habits eg frequency of handwashing or other behavioral factors such as a higher rate of male smokers compared to female smokers.[18,25,33] Smoking was not assessed in our study.

Other Recent SA and MRSA Prevalence Studies from Ecuador

The present study was performed in 2015 and there is hardly any information concerning MRSA prevalence and epidemiology in Ecuador since that time. A couple of publications, all in the Spanish language, demonstrated that MRSA prevalence is probably on the rise.[34] Concerning colonization, a study in 2017 at a speciality hospital in Quito with 191 HCWs from the departments of neonatology, operating rooms, intensive care, and traumatology revealed that 12.5% of the participants carried MRSA, with the highest prevalence of carriage (31%) found among the nursing staff.

Concerning infection, at a rural hospital in a tropical region in Ecuador, a retrospective study published in 2018 identified 235 bacterial isolates from infected wounds. It discovered that ninety-two (39.1%) isolates were SA, of which forty-two (44.7%) were MRSA.[35] Another study, searching for MRSA in 132 clinical SA isolates from blood, skin or soft tissue infection and respiratory samples from patients in Quito, showed that 47% of the strains were MRSA isolates.[36] Moreover, a study from 2019 into guinea pigs in Ecuador raised as livestock revealed that 6.25% of them carried MRSA in the nasopharynx and therefore these animals may potentially play a role in the

transmission of MRSA.[37] These data all together show that MRSA is most probably an underestimated health problem in Ecuador.

Conclusions

Healthy MRSA carriers represent a continuing risk for nosocomial infections and hospital outbreaks. Continuously monitoring and treating HCWs is necessary in order to reduce these risks. In Ecuador, there is a need for consensus recommendations for regular SA carriage screening as well as for decolonization strategies. Surveillance in health staff at reference hospitals in developing countries is essential in order to avoid outbreaks. Direct identification using qPCR is an efficient and rapid option for the surveillance of antimicrobial resistance within a hospital environment, and provides adequate data that can facilitate response and decision-making at the public health level in Ecuador. Moreover, the LightCycler® MRSA Advanced Test was an effective method for screening MRSA in a population that needs continuous and rapid diagnosis and control eg hospital staff.[22,26,38,39] Phenotypic techniques often present difficulties when identifying this type of resistance (22, 33) and molecular tools provide sufficiently sensitive monitoring of SSCmec genes to achieve more accurate surveillance of resistance.

Limitations of This Study

The present study has several limitations. First, the study was performed at a single hospital and the results may not be representative for other hospitals in Quito and are even less likely to represent Ecuador as a whole, given that the country has vastly different climate zones, such as the tropical coast with its largest city, Guayaquil. The nares, throat and perineum are the most prevalent sites for carriage in the general adult population. We sampled only one nostril and found a SA prevalence of 25%. Interestingly, there are reports that show higher carriage rates in, for example, the throat than in the nares when sampled in parallel.[40] Sensitivity may also have been increased by sampling both nares and, for instance, the axillae. No complementary drug resistance was determined in this study, including resistance to fluoroquinolones, aminoglycosides or macrolides.

The study was executed in 2015 and is, as far as we know, with exception of some small published studies in the Spanish language, the most recent study of its kind undertaken in Ecuador, hence the need for updated information. Additional studies are necessary to further characterize MRSA colonization in the general population and among HCWs in different regions of Ecuador as well as further evaluate infection control practices to help prevent MRSA infection and colonization. The sample period was long (one year), which could have influenced the results. However, positive SA and MRSA samples were equally spaced along the sample period and no outbreaks of MRSA infection were reported in the hospital during that time period.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest.

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