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¹Universidade de São Paulo, Faculdade de Medicina, Hospital das Clínicas, São Paulo, São Paulo, Brazil

²Universidade de São Paulo, Instituto de Medicina Tropical de São Paulo, São Paulo, São Paulo, Brazil

³Universidade de São Paulo, Faculdade de Medicina, Hospital das Clínicas, Grupo De Controle de Infecções Hospitalares, São Paulo, São Paulo, Brazil

⁴Universidade de São Paulo, Faculdade de Medicina Veterinária e Zootecnia, São Paulo, São Paulo, Brazil

⁵Universidade de São Paulo, Faculdade de Medicina, Hospital Universitário, São Paulo, São Paulo, Brazil

⁶Universidade de São Paulo, Faculdade de Medicina, Hospital das Clínicas, Instituto de Medicina Física e de Reabilitação, São Paulo, São Paulo, Brazil

Correspondence to: Taniela Marli Bes Universidade de São Paulo, Instituto de Medicina Tropical de São Paulo, Rua Dr Enéas Carvalho de Aguiar, 470, CEP 05403-000, São Paulo, SP, Brazil

E-mail: tanielabes@gmail.com

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Prevalence of methicillin-resistant *Staphylococcus aureus* colonization in individuals from the community in the city of Sao Paulo, Brazil

Taniela Marli Bes^{1,2}, Roberta Ruedas Martins², Lauro Perdigão^{1,3}, Diego Mongelos², Luisa Moreno⁴, Andrea Moreno⁴, Gerson Salvador de Oliveira^{5,6}, Silvia Figueiredo Costa^{1,2}, Anna Sara Levin^{1,2,3}

ABSTRACT

Staphylococcus aureus (SA) is a commensal habitant of nasal cavities and skin. Colonization by community-acquired methicillin-resistant SA (CA-MRSA) is associated with infections in patients who have not been recently hospitalized. The aim of this study is to determine the prevalence of MRSA colonization in an outpatient population, currently unknown in Brazil. Three-hundred patients or caregivers from two teaching hospitals were included. A questionnaire was applied and nasal swabs were obtained from patients. Swabs were inoculated in brain heart infusion (BHI) with 2.5% NaCl and seeded in mannitol. Suspicious colonies were subjected to MALDI-TOF MS MicroflexTM identification. Antimicrobial susceptibility test for oxacillin was performed for SA-positive samples by microdilution. Polymerase chain-reactions for detection of mecA and coA genes were performed for resistant samples. Data about MRSA carriers were compared with non-carriers. There were 127 S. aureus isolates, confirmed by MALDI-TOF. Only seven (2.3%) were MRSA and positive for mecA and coA genes. Factors associated with MRSA carriage were African ethnicity, skin diseases or antibiotic use. The majority of them were from Dermatology clinics. Prevalence of MRSA colonization in individuals from the community was low in our study (2.3%). This finding raises the hypothesis of inter-household transmission of SA, although we did not find any association between MRSA-colonization and the shared use of personal objects. Given the low prevalence of MRSA carriers observed, empirical antimicrobial coverage for MRSA in community-acquired infections should be not necessary.

KEYWORDS: Staphylococcus aureus. MRSA. Skin colonization.

INTRODUCTION

Staphylococcus aureus (SA) is a human commensal and inhabits nasal cavities and skin. It is estimated that 30-60% of the population are transiently colonized and 20% carry SA persistently^{1,2}.

Although originally a nosocomial pathogen, methicillin-resistant SA (MRSA) has caused community-acquired infections since the 2000s³. The first community-acquired MRSA (CA-MRSA) report was in Australia in 1993, in local indigenous people, and since then outbreaks have been described in different contexts. In Brazil CA-MRSA was reported sporadically⁴⁻⁷.

CA-MRSA is associated with infections in patients with no recent history of hospital admission and does not appear to be related to the classic risk factors for MRSA colonization⁸. Risk factors for CA-MRSA are not well established, and

include: living in agglomerations, children, antibiotic use for less than one year, chronic skin disease, HIV infection, and low socioeconomic level^{1,9,10}.

Currently the prevalence of MRSA colonization in the community is unknown in Brazil.

The objective of this study was to determine the prevalence of MRSA-colonized individuals in a population from an outpatient healthcare facility.

MATERIALS AND METHODS

Outpatients and persons who accompany them at two hospitals were included in the study during the period from January to April 2017. Hospital das Clinicas (HC) is a tertiary-care facility and Hospital Universitario (HU) is a community hospital. Both are teaching facilities affiliated with the University of Sao Paulo, Brazil. The research was conducted after the approval of the Research Ethics Committees of both hospitals (N° 1.887.780 and 1.930.074). Data were consolidated and analyzed, and patients' identities were confidential.

Inclusion criteria: Individuals with at least 18 years of age who passed the entrance doors of the outpatient Infectious Diseases and Dermatology Clinics at HC, emergency room, at both hospitals and the immunization center.

Exclusion criteria: history of use of long-term catheters, undergoing surgery or hospitalization within the last year.

Individuals included in the study, after signing the consent form, answered a questionnaire on personal data; demographics; daily habits; sports; personal hygiene; the presence of pets at home; illicit drug use; family members living in the same household; public transport (bus, subway or train); trips in the last year; frequency at motels, gyms, swimming pools or other crowded places; history of diseases such as Aids, cutaneous conditions, sexually transmitted diseases and use of antibiotics in the last year.

The number of subjects to be included in the study was calculated taking into account a population of 10 million inhabitants, a prevalence of MRSA colonization of 2% in this population, a 95% confidence interval with a 5% variation. The sample was composed of 300 subjects, divided equally between the five sectors, with 60 each.

For sample collection, swabs (COPAN Venturi Transystem®, COPAN Diagnostics Inc., Murrieta, California, USA) were used. Samples were collected from the anterior nares by gently rotating the swab in each nare. One sample was obtained from each subject. Swabs were initially inoculated in Brain Heart Infusion medium (BHI) with 2.5% NaCl to stimulate the growth of cutaneous agents, incubated for 24 h and then seeded on regular mannitol

agar. Samples that converted mannitol agar to yellow staining were subjected to MALDI-TOF MS MicroflexTM identification (Bruker Daltonics, Billerica, Massachusetts, USA). The criteria for interpreting the results were: scores = 2.0 were accepted for species assignment and scores = 1.7 and <2.0 were used for genera identification.

SA isolates were submitted to antimicrobial susceptibility testing by microdilution for oxacillin¹¹. Samples were tested in duplicate. Isolates identified as resistant to oxacillin were submitted to Polymerase Chain Reaction for *mec*A and *co*A genes for confirmation.

Analysis of data: individuals colonized with MRSA strains from the community and without hospitalization within the past year were compared with non-colonized individuals. For the analysis of the dichotomous variables, non-parametric chi-square test and Fisher's exact test were used. Numerical variables were analyzed using the Student T test. Also, means and standard deviations were presented.

Statistical analysis was performed with the Epi Info v3.1.3 (StatCalc Statistical Calculators).

RESULTS

Three hundred samples were collected from patients and accompanying persons: 61% were female and 95% were heterosexual. None were military in the last year; two had been incarcerated at some point during their lives but had been free for more than one year. None were intravenous drug users or lived in the same household as a drug user. Eight reported sharing a home with marijuana users, three with inhaled cocaine users andone with volatile product inhalers. Two were tobacco smokers.

Of the 300 samples collected, 127 were positive for *S. aureus* of which seven had a MIC = $4 \mu g/mL$ (4-128) and were positive for *coA* and *mecA* genes.

Five of the seven MRSA isolates were collected from patients or persons that accompanied patients at the Dermatology outpatient clinic. When comparing MRSA carriers with non-carriers, the Dermatology unit was associated with MRSA colonization (OR: 10.81; 95% CI: 2.04 - 57.23 p: 0.0042). Other factors associated with MRSA carriage were: history of skin disease; skin color; use of motels. The use of antibiotics in the last year was a protective factor for colonization (Table 1).

DISCUSSION

We found a very low prevalence of MRSA colonization in individuals from the community: 2.3% of the entire population and 5.5% among the persons colonized by *S. aureus*.

Table 1 - Univariate analysis of characteristics associated with methicillin-resistant *Staphylococcus aureus* colonization among persons visiting outpatient clinics or emergency rooms or vaccination clinics

Characteristics	Negative for MRSA n= 293	MRSA n=7	OR (odds ratio)	IC (95%)	p value
Male	113 (39%)	3 (43%)	1.247	0.274-5.679	1
Heterosexual	277 (95%)	7 (100%)	0	0	1
Black ethnicity	56 (19%)	4 (57%)	5.642	1.226-25.93	0.0316
Divides room with 1 or more people	228 (78%)	5 (71%)	0.726	0.137-3.832	0.658
Has pets in general	140 (48%)	5 (71%)	2.732	0.521-14.308	0.269
Dogs	112 (38%)	4 (57%)	1	0.107-9.301	1
Use of antiseptic soaps	157 (54%)	3 (43%)	0.649	0.142-2.954	0.709
Practices sports	122 (42%)	2 (28%)	0.56	0.107-2.937	0.703
Use of Shared Objects:					
Shaver	39 (13%)	1 (14%)	1.085	0.127-9.26	1
Soap	155 (53%)	5 (71%)	2.225	0.425-11.657	0.455
Bath sponge	141 (48%)	4 (57%)	1.437	0.316-6.535	0.715
In the last year:					
Family history of surgical procedure	31 (11%)	1 (14%)	1.408	0.164-12.086	0.549
Attended motels	20 (7%)	3 (43%)	10.237	2.142-48.9292	0.011
Beach trips	113 (39%)	4 (57%)	2.123	0.466-9.665	0.437
History of skin diseases	35 (12%)	3 (43%)	5.528	1.187-25.738	0.045
History of antibiotic use	75 (26%)	5 (71%)	7.266	1.38-38.244	0.016
Amoxicillin	41 (14%)	1 (14%)	1.024	0.120-8.729	1
Azithromycin	15 (5%)	2 (28%)	7.413	1.327-41.407	0.053
Sulfamethoxazole + Trimethoprin	4 (1%)	0	0	0	1
Quinolones	10 (3%)	1 (14%)	4.716	0.517-42.952	0.232
N/A	17 (6%)	1 (14%)	2.705	0.308-23.769	0.354
Use of public transportation	233 (80%)	7 (100%)	0	0	0.351
Bus	167 (57%)	6 (86%)	4.526	0.538-38.079	0.245
Place of collection					
Dermatology outpatients clinic	55 (19%)	5 (71%)	10.818	2.044-57.23	0.0042
Emegency room - HU	59 (20%)	1 (14%)	0.661	0.078-5.597	1
Emegency room - HC	59 (20%)	1 (14%)	0.661	0.078-5.597	1
Dermatology outpatients clinic	60 (20%)	0	0	0	0.351
Imunization center	60 (20%)	0	0	0	0.351

The prevalence of MRSA causing cutaneous infections in patients seeking emergency services in the US varied widely from 15% to 74% in New York and Kansas City, respectively¹². Over the years, the incidence of CA-MRSA infections in the United States has increased from 2.5% to 39%, with clonal heterogeneity in different regions¹³⁻¹⁵.

A published European study involving England, France, Germany, Italy, Greece, Romania and Spain included 205 cases of skin and soft tissue infections¹⁶. The mean

prevalence of MRSA was 15.1%, with no cases in Northern countries and 29% in Southern countries.

In Brazil, community-acquired MRSA cases have been reported in the cities of Porto Alegre, Rio de Janeiro, Botucatu and Bahia. All cases have in common cutaneous involvement and the presence of PVL toxin^{6,17,18}. A study evaluated nasal carriage of MRSA¹⁹ among 250 first and second year students from University of Londrina, in the South of the country, with a prevalence of 2.5%, a similar result to our study. Among 148 children in a daycare center

in Northeastern Brazil, MRSA nasal carriage was 7.4%²⁰.

A previous study from our group showed that among hospitalized patients in the Dermatology Unit MRSA carriage was 45%, of which a quarter were community-acquired²¹. Among chronic dermatologic patients, MRSA carriage is frequent, and there may be household transmission explaining the colonization of the people who accompanied patients to the Dermatology outpatient clinic in our study, although we did not find a relationship with statistical significance between colonization by MRSA and the shared use of personal objects.

Other findings in our study such as black ethnicity have been previously described by Tosas Auguet *et al.*²², but we do not know how to explain it. History of antibiotic has been previously described by Gustave *et al.*²³, who suggested that previous antibiotics use contributed to the increase of MRSA lineages in the community. The association between attending motels and MRSA colonization has not yet been described and is difficult to explain. It may be due to the collective environment and objects shared by multiple individuals.

Due to the low prevalence of colonization found in our sample, we suppose that empirical coverage against MRSA, with drugs such as clindamycin and sulfamethoxazole-trimethoprim (SMX-TMP) or even nasal decolonization with mupirocin, as recommended by the IDSA²⁴ guideline can be used in patients from the community without risk factors, in our setting.

The IDSA guideline made the US recommends the use of MRSA coverage for patients with severe purulent or non-purulent infection (patients with abscesses, signs of sepsis or impaired immune status), skin and soft tissue infection from the community. However, this recommendation is based on studies performed in settings different from ours, with high MRSA prevalence in the community.

Our study has limitations. Our sample was obtained in hospitals and may not represent the community. However, if so, the prevalence of MRSA may be even lower than the one we observed. The study was carried out in two hospitals in the city of Sao Paulo, not allowing extrapolation of data to the entire State or even to other regions of the country. Furthermore, due to the study design, we may have included temporary nasal carriers. On the other hand, our sample was very heterogeneous, which may allow us to generalize our results and suggest empirical therapy for infections in which *S. aureus* is suspected.

CONCLUSION

Given the low prevalence of MRSA nasal carriage found in our study (2.3%), we believe that empirical coverage for MRSA is not necessary for patients with community-acquired infections caused by *Staphylococcus aureus*.

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