Nasal carriage of methicillin-resistant *Staphylococcus aureus* in university students

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ABSTRACT

In a study of university students, the percentage nasal carriage of *Staphylococcus aureus* was 40.8% (102/250). Of the isolates, MIC_{50} of methicillin was 0.5 μg/mL and MIC_{90} was 1 μg/mL. Six (5.8%) isolates were methicillin-resistant and carried the *mecA* gene. These results suggest that community-associated methicillin-resistant *S. aureus* may be spreading in Brazil.

Keywords: nasal carriage, CA-MRSA, *S. aureus.*

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Methicillin-resistant *Staphylococcus aureus* (MRSA) is an important cause of health care-associated infections worldwide.[1] In recent years, cases of MRSA infection have been reported more frequently in healthy community individuals with no traditional risk factors for MRSA infection.[2] These infections, apparently acquired in the community, are termed community-associated MRSA infections.[2,3]

Community-associated MRSA (CA-MRSA) strains differ from health care-associated (HA) MRSA strains in terms of epidemiology, microbiology, and clinical manifestations.[3] CA-MRSA strains are generally susceptible to most antibiotics, contain staphylococcal chromosome cassette *mecA* type IV, produce the virulence factor Panton-Valentine leukocidin, and cause mainly skin and soft-tissue infections.[2,4]

It is well recognized that nasal carriage of MRSA represents a major risk factor for subsequent infection and transmission of this pathogen.[5,6] Although several studies have reported the prevalence of MRSA nasal carriage in patients in health care-settings,[7,8] this subject has been little investigated in healthy individuals in the broader community,[9] and is practically unstudied in Brazil.

In this study, we determined the prevalence of nasal carriage of *S. aureus* in university students. Antibiotic susceptibility patterns, minimal inhibitory concentration to methicillin, and *mecA* gene detection of the isolates were included.

Volunteers – The volunteers were 250 healthy adults, 93 males and 157 females, including first- and second-year undergraduate students in pharmacy, nursing, dentistry and medicine at a Brazilian university. Their ages ranged from 18 to 27 years. After giving their written informed consent, the volunteers completed a questionnaire to obtain demographic data and information on risk factors for MRSA infection. The study was approved by the Institutional Review Board of the State University of Maringá.

Specimen collection – Each volunteer had one nasal specimen collected from the anterior nares, with a dry swab (Copan Diagnostics, Corona, CA, USA). Swabs were transported in Amies medium and processed within 2 h of collection. All swabs were collected by the same investigator during the study, which was conducted from February to June 2007.

Culture methods – The swab was first inoculated directly on mannitol-salt agar (MSA) (Difco Laboratories, Sparks, MD, USA). It was then placed in a tube containing 10 mL of tryptic soy broth (TSB) (Difco) supplemented with 7.5% sodium chloride (enrichment broth), which was subcultured on MSA after 24 h. Inoculated plates were screened at 24, 48, and 72 h for typical colonies of staphylococci. *S. aureus* was identified by colonial morphology, Gram stain, and tube coagulase test.

Antimicrobial susceptibility testing - The antimicrobial susceptibility of *S. aureus* isolates was

assessed by disk-diffusion tests, according to the Clinical Laboratory Standards Institute guidelines.[10,11] The following antimicrobial disks were used: penicillin (10 U), oxacillin (1 µg), ciprofloxacin (5 µg), gentamicin (10 µg), amikacin (30 µg), telithromycin (15 µg), linezolid (30 µg), trimethoprim-sulfamethoxazole (1.25-23.75 µg), and vancomycin (30 µg). All the disks were obtained from Oxoid (Oxoid, Basingstoke, UK). A mupirocin (5 µg) disk (Oxoid) was also tested, according to Finlay *et al.* (1997).[12]

Minimal inhibitory concentration (MIC) – MIC to oxacillin was assessed by both E-test® oxacillin strips (AB-Biodisk, Solna, Sweden) according to the manufacturer's instructions, and the agar dilution method as recommended by the Clinical Laboratory Standards Institute.[11,13]

Detection of mecA by polymerase chain reaction (PCR) – One hundred and two strains of *S. aureus* were analyzed by PCR assay, using total DNA after boiling the bacterial cells. The *mecA* gene was detected using *mecA* sense 5'TGGCTATCGTGTCACAATCG3' and *mecA* antisense 5'CTGGAACTTGTTGAGCAGAG3' primers. Cycling parameters were 94° C for 5 min followed by 30 cycles of 94° C for 30 sec, annealing at 52° C for 30 sec, extension at 72° C for 30 sec, and a final 7 min incubation at 72° C. The amplification products (309 bp) were analyzed by electrophoresis in 1% agarose gel stained with ethidium bromide.

S. aureus was isolated from nasal swabs of 102 (40.8%) of the 250 volunteers, and six (2.4%) of them were CA-MRSA carriers. None of the volunteers had any identified risk factor.

Recovery of *S. aureus* strains from volunteers' nostril by direct plating on MSA was 26.8% (67 of 250), and by enrichment broth was 40.8% (102 of 250). All the volunteers detected as nasal carriers of *S. aureus* by the MSA method, were also detected by the enrichment broth method.

All the 102 strains of *S. aureus* were sensitive to vancomycin, telithromycin, linezolid, gentamicin, and trimethoprim-sulfamethoxazole. Resistance to penicillin G, ciprofloxacin, oxacillin, and amikacin was found in 92.0%, 8.8%, 5.8%, and 4.0% of the strains, respectively. Mupirocin resistance was detected in 6 of 102 (5.8%) isolates, which were sensitive to oxacillin.

Of the 102 strains, the minimal inhibitory concentration to oxacillin ranged from 0.06 µg/mL to 256 µg/mL, and MIC_{50} and MIC_{90} were 0.5 µg/mL and 1.0 µg/mL, respectively. Six strains (5.88%) showed MIC µ 32 µg/mL and carried the *mecA* gene, and were therefore considered CA-MRSA. Two CA-MRSA strains were recovered only by the enrichment broth method. E-test® and agar dilution methods gave similar results.

The prevalence of *S. aureus* nasal carriage varies according to the quality of sampling, culture techniques, and the population studied.[5,9] Early cross-sectional surveys on nasal carriage demonstrated a mean carriage rate of 37.2%.[5] More recent studies have reported rates of approximately 27% in healthy adult populations.[6,14] Two studies with pre-clinical medical students showed that 35.2% and 29% were *S. aureus* nasal carriers.[15,16] Our results are consistent with these findings.

Although CA-MRSA emerged as a cause of infection in the community in the 1990s, the first report of infections caused by this microorganism in Brazil was published in 2005.[17] Our study found the prevalence of CA-MRSA nasal carriage in our student community to be 2.4%, which is higher than the findings of similar studies by investigators outside Brazil.[9,16,18]

In cross-sectional studies, the choice of the microbiological method used for *S. aureus* carriage detection is important, because the nasal culture is done only once. The enrichment broth method has been recommended to increase the sensitivity of detection of MRSA carriage.[19] In our study, two of the six CA-MRSA strains were isolated only by the enrichment broth, and the use of this method resulted in improved recovery rates of 14%.

As expected, all the *S. aureus* isolates, including the six CA-MRSA, were susceptible to most of the antimicrobial agents tested. Although most of these strains were sensitive to oxacillin, their MIC_{50} and MIC_{90} were near the resistance breakpoint to oxacillin (i.e., MIC ≥ 4 µg/mL), suggesting less potency and antimicrobial activity of this drug. This finding may be important for developing therapies for staphylococcal diseases.

In respect to mupirocin, the heavy growth with no visible zone of inhibition around the mupirocin disk observed in six *S. aureus* isolates may indicate a high level of resistance (i.e., MIC ≥ 1,024 µg/mL) to this drug.[20] This fact is worrisome because mupirocin is widely used for prevention of *S. aureus* intranasal colonization.[1]

Our results showed that all strains resistant to oxacillin by phenotypic methods carried the *mecA* gene. This was not observed in the oxacillin-sensitive *S. aureus* strains.

In conclusion, the results of this study showed that *S. aureus* nasal colonization is common in our student community, and suggest that CA-MRSA may be spreading in Brazil.

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