

The nares as a CA-MRSA reservoir in the healthy elderly

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ABSTRACT

Introduction: The frequency of methicillin-resistant *Staphylococcus aureus* (MRSA) has increased in the community. This study evaluated the prevalence of MRSA and community-acquired (CA)-MRSA in 120 healthy elderly. **Methods:** The MRSA were evaluated for the presence of the IS256, *mecA*, *agr*, *icaA*, *icaD*, *fnbB*, and *pvl* genes with PCR. **Results:** Frequency of *S. aureus* and MRSA colonization was 17.8% and 19%, respectively. CA-MRSA isolate showed SCCmec IV, *fnbB*⁺, and *icaD*⁺. **Conclusions:** CA-MRSA was detected, with genotype determined as SCCmec type IV/IS256/*fnbB*⁺/*icaA*/*icaD*⁺/*bbp*/*agr2*/*bap*/*pvl*, characterizing this population as a possible reservoir of this organism in the community.

Keywords: CA-MRSA. Elderly. Reservoir.

Over the past decade, Methicillin-resistant *Staphylococcus aureus* (MRSA) has been a major clinical and epidemiological problem in hospitals. It is reported with increased frequency in community populations without prior health care contact⁽¹⁾. Community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) is susceptible to the majority of non-beta-lactamic antibiotics. It frequently expresses more virulent genes as *pvl* and predominantly carries the SCCmec type IV⁽²⁾.

In colonized individuals, nasal carriage of MRSA acts as a potential endogenous reservoir for clinical infections or as a source of cross-colonization for spread in the community⁽³⁾. We conducted a cross-sectional study from November 2013 to February 2014 at the Faculty of Physical Education, Federal University of Uberlândia, and looked for MRSA in the anterior nares of 120 healthy elderly (≥ 60 years) people who practiced regular physical activity. The research protocols were approved by the institutional review board, and all volunteers signed informed consent forms before the study began.

Using sterile swabs pre-moistened with Tryptic Soy Broth (Becton Dickinson, Le Pont de Claix, France) we collected bacterial samples from the nares. After collecting, swabs were promptly transported to the Laboratory of Microbiology and

incubated at 35°C, overnight. About 0.1ml of this suspension was inoculated onto the surface of petri dishes selected (Mannitol Salt Agar - Himedia, India) for growing *S. aureus*. The bacterial isolates were identified with phenotypic classical tests (isolates were tested for the production of the coagulase enzyme via the tube method, which indicates *S. aureus* and separates it from the commonly isolated negative coagulase *Staphylococcus*). The MRSA and the susceptibility profile to several antimicrobials (oxacillin, 1µg; cefoxitin, 30mg; ciprofloxacin, 5µg; clindamycin, 2µg; gentamicin, 10µg; tetracycline, 30µg; rifampicin, 30µg; erythromycin, 15µg and sulfamethoxazole/trimethoprim, 25µg) were confirmed with the disk diffusion method using Mueller Hinton Agar (Himedia, India). The cefoxitin-resistant isolates according to disk diffusion were confirmed based on minimum inhibitory concentration (MIC) for oxacillin by performing the E-test[®] (AB Biodisk, Solna, Sweden) and the presence of *mecA* with polymerase chain reaction (PCR). *Staphylococcus aureus* ATCC 25923 was preferred as a quality control strain⁽⁴⁾.

Purification of genomic deoxyribonucleic acid (DNA) of four isolates characterized as MRSA by disk diffusion was prepared using QIAamp DNA Mini Kit (Qiagen) according to the manufacturers recommendations. The *pvl*, *icaA*, *icaD*, IS256, *agr*, *bap*, and *fnbB* genes were assessed with end-point PCR according to methods described by Saïd-Salim et al.⁽⁵⁾, Martin-Lopez et al.⁽⁶⁾, Arciola et al.⁽⁷⁾, Gu et al.⁽⁸⁾, Shopsin et al.⁽⁹⁾, Cucarella et al.⁽¹⁰⁾ and Tristan et al.⁽¹¹⁾. The oligonucleotide primers described previously by Kondo et al.⁽¹²⁾ were used for verifying the various types of SCCmec elements. *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228 and Clone Southwest Pacific were preferred as a quality control strain.

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TABLE 1 - Characteristics of colonized elderly people analyzed in the study.

Characteristics *	Nares colonization (n= 118)**		Nares colonization by <i>Staphylococcus aureus</i> (n= 21)	
	n	%	n	%
Sex				
female	90	76.3	17	81.0
male	17	14.4	2	9.5
Age (years)				
60 –70	56	47.5	13	61.9
71 – 80	40	33.9	6	28.6
≥ 81	11	9.3	0	0.0
Marital status				
married	49	41.5	11	52.4
single	18	15.3	6	28.6
widower	40	33.9	2	9.5
Residents in the household				
1 – 4	98	83.1	16	76.2
5 – 8	9	7.6	3	14.3
Time physical activity ≥ 150 min per week	71	59.2	16	76.2
Hospital procedure and/or hospitalization in the past 12 months	19	15.8	3	14.3

*It was not possible to collect information from 11 elderly individuals. **118 nares colonized of the 120 collected.

Table 1 shows that a total of 21 *S. aureus* isolates were recovered from the nares of 120 subjects, and the nasal carriage rate was 17.8% (21/118 nares colonized by *Staphylococcus* spp.). The disk diffusion method characterized 19% (4/21) of the isolates as MRSA and 50% (2/4) with MIC ≥4mg/L (only one multidrug resistance isolate). In total, 50% of the MRSA showed multidrug resistance with resistance to erythromycin, rifampicin, and clindamycin. On the other hand, all strains of MRSA were susceptible to ciprofloxacin, gentamicin, amikacin, tetracycline, and sulfamethoxazole/trimethoprim.

The presence of the *mecA* gene was confirmed in two isolates with MIC ≥4mg/L, which carried SCCmec type II and IV, respectively. Despite not being tested, it is possible that the other two samples detected in our search (*mecA*⁻, cefoxitin resistant) have the new homologous variant *mecC* that has 70% identity with *mecA*. The *mecC* gene is not detected with the PCR method established for the detection of *mecA*; consequently, isolates harboring this new variant can be misidentified and reported as methicillin-susceptible *S. aureus*⁽¹³⁾. The CA-MRSA isolated showed the following genotypic profile: SCCmec type IV/IS256-/f_{nb}B⁺/icaA⁻/icaD⁺/bbp⁻/agr2/bap⁻/pvl⁻. None of the elderly participants colonized with MRSA had previous hospitalization or had undergone any hospital procedure in the 12 months before the study.

The majority of studies conducted to evaluate asymptomatic carriage of MRSA have been performed in individuals in the

community who have had contact with a hospital environment. Studies of epidemiological surveillance for CA-MRSA carriage in healthy subjects are rare in Brazil. This study provides the first description of CA-MRSA with genotypic profile in this population.

In our study, we also detected a strain carrying the SCCmec type II. The epidemiology of MRSA is a dynamic process that brings to light the blurring of the limits between the community and hospital⁽¹⁴⁾. According to the literature, the strains carrying SCCmec type II are associated with clinical isolates recovered from the hospital⁽²⁾. One possible explanation for this is that the elderly participants have probably had previous contact with someone who was recently hospitalized. Additionally, previous contamination of the environment is also considered an important reservoir of microorganisms⁽¹⁴⁾.

Although we have found only one SCCmec type IV *pvl*⁻, the frequency of nares colonized by MRSA (19%, 4/21) were similar to those found in Mexico City, where 12.6% of *S. aureus* carriers were colonized with MRSA, both rates higher than those reported in the United States (1.5%)⁽³⁾.

We compared PCR assays for the detection of the methicillin-resistance gene with classical methods in this study. The two isolates initially classified as methicillin-resistant based on disk diffusion showed discrepant results when tested with the E-test[®] and PCR. Investigators have reported that the conventional susceptibility testing methods, mainly the diffusion

disk technique, are not always reliable for methicillin-resistant staphylococci detection⁽¹⁵⁾.

Molecular epidemiology studies have highlighted the global spread of different MRSA clones. HA-MRSA may have a hidden reservoir in the community just as the CA-MRSA has one in health services. This increases the need to monitor such clones⁽¹⁴⁾. Although our isolates were not clonally typed, the presence of CA-MRSA and HA-MRSA genotypes colonizing healthy elderly participants in a multisport center emphasizes the risk of cross-transmission. The presence of different MRSA clones in this group could not be determined in our study, but warrants additional research.

In this study of healthy elderly people in Minas Gerais, Brazil, we detected the prevalence of MRSA, including CA-MRSA, among *S. aureus* carriers. The importance of CA-MRSA colonization as a risk factor for cross-transmission in the community must be further analyzed.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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