Original Article

Evaluation of methicillin-resistant *Staphylococcus aureus* nasal carriage in Malagasy patients

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Abstract

Introduction: Methicillin-resistant *Staphylococcus aureus* (MRSA) is an important cause of infections. It is well recognized that nasal carriage of *S. aureus* represents a potent and increasingly prevalent risk factor for subsequent *S. aureus* infection. However, in Madagascar no data exist concerning this nasal carriage of *S. aureus*.

Methodology: Nasal swabs from 304 different patients attending the Laboratory of Training and Research in Medical Biology of Madagascar were cultured for methicillin sensitive (MSSA) and MRSA.

Results: One hundred and sixteen patients had *S. aureus* in their noses (38.16 ± 5.46%) of whom 45 (14.80 ± 3.99%) had MRSA. A risk factor for MSSA nasal carriage included a history of hospitalization when antibiotics were administered (odds ratio [OR] 2.25, 1.09 - 4.64). Among MRSA nasal isolates, high rate of resistance to other antibiotics was observed, particularly for trimethoprim-sulfamethoxazole (68.89%), erythromycin (66.67%) and ofloxacin (53.33%).

Conclusion: Our data showed a high rate of MRSA nasal carriage and a high rate of multidrug resistance. A strategic policy against the spread of multidrug resistant strains is desirable.

Key words: MRSA; Malagasy lab; nasal carriage; risk factors

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Introduction

S. aureus colonizes naturally the skin and nasal mucosa of human beings [1]. Cross-sectional surveys of healthy adult populations have reported *S. aureus* nasal carriage rates between 20% and 55% [2], and longitudinal studies showed that about 20% (range12% to30%) of individuals are persistent *S. aureus* nasal carriers while approximately 30% are intermittent carriers (range 16% to 70%) [3,4]. This colonization is with methicillin-susceptible *S. aureus* (MSSA). However, methicilin-resistant *S. aureus* (MRSA) can colonize healthy people at a lower rate, about 1% to 8%, and represents a potent and increasingly prevalent risk factor for subsequent *S. aureus* infection [5]. There is a worldwide increase in the number of infections caused by MRSA, but according to a study conducted at the Pasteur Institute of Madagascar, the prevalence of MRSA in *S. aureus* infection in the Malagasy community is still very low (5.8 %) [6]. However, data concerning the frequency of nasal carriage of *S. aureus* in the Malagasy community is not known. It is important to determine the prevalence of *S. aureus* nasal carriage because it can influence antibiotic therapy decisions.

In the present study, we assessed *S. aureus* nasal carriage in patients coming for various medical analyses to the Laboratory of Training and Research in Medical Biology of Madagascar, to estimate MSSA and MRSA colonization and to identify some colonization risk factors in a section of the Malagasy community.

Methodology

Sampling procedures

Three hundred and four different patients coming for various analyses in our laboratory were asked to consent to a nasal swab of their anterior nares for culture of *S. aureus*. Participants completed a very brief questionnaire asking age, sex, previous hospitalization and antimicrobial use. All the patient samples and questionnaires were collected within a two-month period.

Identification and antimicrobial susceptibility of S. aureus

The single swab from each patient was immediately inoculated in Columbia blood agar 5% and incubated for 24 hours at 37°C. Plates were examined after 24 hours, and isolates were identified as *S. aureus* by their colony morphology, Gram-positive stain, positive catalase reaction, and positive tube coagulase assay. *S. aureus* isolates were inoculated onto Baird Parker agar with Rabbit Plasma Fibrinogen bovin (Conda-Pronadisa, Madrid, Spain) for isolation of MSSA and onto selective chromogenic MRSA agar supplemented with 4 µg/mL of cefoxitin from Conda-Pronadisa for isolation of MRSA. Coagulase-positive reactions of isolates were confirmed by the presence of black colonies ringed with a precipitation halo on Baird Parker agar [7], and methicillin resistance was confirmed by the demonstration of blue colonial growth on selective chromogenic MRSA agar [8]. Susceptibility of MRSA to eight antibiotics (oxacillin, penicillin, erythromycin, lincomycin, ciprofloxacin, tetracycline, trimethoprim-sulfamethoxazole, gentamicin) was assessed by disc diffusion technique following the guidelines of the Antibiogram Committee of the French Society for Microbiology (CASFM) [9]. Briefly, an inoculum of 10^6 CFU/mL was prepared and seeded in a Mueller-Hinton square plate. After incubation for 24 hours at 37°C, the inhibition zones around the antibiotic disks (BioRad, Marnes-la-Coquette, France) were measured. For susceptibility to oxacillin, inocula of 10^7 CFU/mL were prepared and plates were incubated at 37°C for 24 hours on Mueller-Hinton agar with 2% NaCl. Breakpoints for resistance were those recommended by the CASFM [9]. Duplicate testing was performed on 30 detected MSSA and MRSA strains. *S. aureus* ATCC 25923 strains were used as a quality control. Multidrug resistance was defined as resistance to penicillin and oxacillin plus two or more antibiotics listed previously.

Statistical analysis

Prevalence and 95% confidence intervals (CIs) were calculated for overall *S. aureus*, MRSA, and MSSA colonization. Categorical comparisons were performed using $\chi 2$ analyses. Logistic regression was used to estimate the associations among age, sex and colonization. $P < 0.05$ was considered significant for all comparisons. Risk factors for *S. aureus* colonization were also evaluated and variables achieving a $P < 0.05$ level were considered significant; odds ratios (ORs) with 95% CIs were calculated using GraphpadPrism5 software (Avenida de la Playa, La Jolla, CA, USA).

Results

Table 1 shows the characteristics of patients colonized by Staphylococcus aureus and methicillin-resistant *S. aureus*. Over the course of two months, our laboratory received samples from 1,430 individuals for

Table 1. Characteristics of patients colonized by *Staphylococcus aureus* and methicillin-resistant *S. aureus* (MRSA)

Characteristic	All participants (n=304)	With *S. aureus* result test n (%)			With MRSA result test n (%)		
		Negative=188	Positive=116	*p*	Negative=259	Positive=45	*p*
Age, mean ± SD, years	33.47 ± 17.55	32.5 ± 16,5	34.75 ± 18.75		35 ± 16.5	36 ± 17.2	
Male	116 (38.16)	70(37.23)	46 (39.65)		102 (39.38)	14 (31.11)	
Female	188 (61.84)	118(62.76)	70 (60.34)		157 (60.61)	31 (68.89)	
Previous[a] antimicrobial use	90 (29.60)	48 (25.53)	42 (36.20)	.04	71 (27.41)	19 (42.22)	.04
Previous hospitalization	106 (34.86)	57 (30.32)	49 (42.24)	.03	90 (34.74)	16 (35.56)	
Previous antimicrobial use with previous hospitalization	34 (11.18)	15 (7.98)	19 (16.38)	.02	30 (11.58)	4 (8.88)	

[a] prior 6 months

various medical analyses. Nasal swabs were collected from 304 (21.25%) consenting patients, with a sex ratio of M/F: 0.61 with a mean ± SD age of 33 ± 17. 55 years. *S. aureus* were isolated from 116 of 304 (38. 16 % ± 5.46%) patients. Seventy-one (61.2% ± 8.8%) individuals were colonized with MSSA, and forty-five (38.8% ± 8.8%) were colonized with MRSA, for an overall estimate of MRSA colonization prevalence of 14.8%.

No significant association was found between age and MSSA, MRSA colonization (Figures 1A and 1B) or between age and non-colonized patients (P = 0.67). No association was found between sex and MSSA, MRSA colonized patients or between age and non-colonized patients (P = 0.13). Recent antibiotic use (OR 1.93, 95% CI 1.01 to 3.71; P = 0.04) was identified as being associated with MRSA colonization. History of hospitalization was significantly associated with MSSA colonization and global *S. aureus* colonization (OR 1.68, 95% CI 1.04 to 2.72; P = 0.03). Recent antibiotic use with history of hospitalization (OR 2.25, 95% CI, 1.094.64; P = 0.02) was identified as being associated with MSSA and global *S. aureus* colonization on multivariate analysis. Most MRSA strains (36/45) express heterogeneous character according to the presence of isolated colonies close to the oxacillin disc inhibition zone.

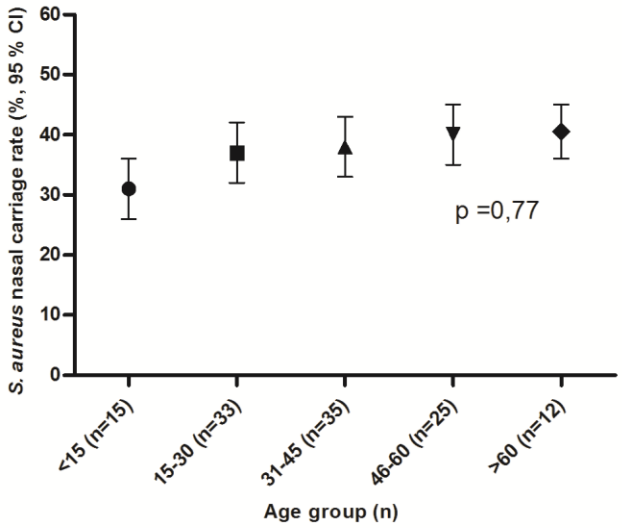
Sixty-four (90.14%) MSSA strains were resistant to penicillin; resistance rates of MRSA to the other tested antibiotics are shown in Table 2. Sixteen MRSA strains were multidrug resistant, of which eight (17.77%) strains were resistant to seven antibiotics and two (4.44%) strains were resistant to all the antibiotics tested (Table 3).

Discussion

This is the first report of *S. aureus* and MRSA nasal colonization among patients, who may or may not have been ill, attending a Malagasy health clinic for various medical analyses. Although the *S. aureus* colonization (38.16%) reported here is similar to that in previous studies, MRSA colonization (14.8%) is higher than previously reported (1% - 8 %) [10]. This rate seems to be similar to populations with high-risk colonization factors such as homelessness [11], hospitalization [12], drug abuse, [13] and exposure to horses [14].

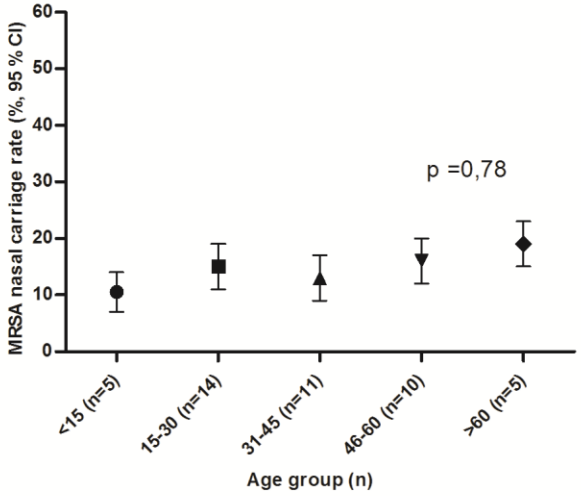
We found that a history of antibiotic use and hospitalization was identified as a risk factor for *S. aureus* and MRSA colonization which was consistent with data reported in other studies [15,16]. Because we did not culture repeat nasal swabs, we could not differentiate between persistent and intermittent carriers. According to the Centers for Disease Control and Prevention (CDC), criteria for categorizing an isolate as a community-acquired MRSA (CA-MRSA) are primarily based on patient history such as no history of MRSA infection, hospitalization, or exposure to a health-care facility within the previous year. In our questionnaire we did not ask for this

Figure 1A: Rate of nasal carriage of *S. aureus* according to age group



n represents total patients of each age group

Figure 1B: Rate of nasal carriage of MRSA according to age group



information so we could not estimate CA-MRSA carriers. We were not able to identify specific hygiene-related practices or health conditions such as frequency of hand washing, showering, or doing laundry that were associated with an increased risk of MSSA or MRSA colonization. Moreover, we did not assess other risk factors such as residence with a health-care worker or veterinary professional, regular participation in group sports activities, previous MRSA infection, or contact with individuals diagnosed with MRSA and regular contact with animals.

As we used an incubation temperature of 37°C instead of 35°C as recommended by the Clinical and Laboratory Standards Institute, the number of resistant *S. aureus* isolates detected may have even been underestimated [17]. However, the high number of MRSA strains detected with the technique used was disquieting.

In comparison with MRSA isolated from different pathological samples reported by the Pasteur Institute of Madagascar [6], our MRSA nasal strains had significantly high rates of resistance to other antibiotics, particularly trimethoprim-sulfamethoxazole (68.89% versus 38.88%), erythromycin (66.67% versus 33.33%) and ofloxacin (53.33% versus 13.88%). This high rate of drug resistance among MRSA isolates is of concern and may be reflective of the large amount of antimicrobials used in our community. In Madagascar, many antimicrobials are available without any medical prescription and used even in non-bacterial infections.

Because we did not determine the genetic subtypes of isolates, we could not determine if our samples were from clusters of individuals colonized by the same bacterial strains. It would be interesting to determine their origin to assess the progressive spread of MRSA Malagasy strains. Additionally, it would be

Table 2. Antibiotic resistance profiles of methicillin-resistant *S. aureus* (MRSA) nasal isolates as determined by disk diffusion

Antibiotics	MRSA (n = 45) n (%)
Penicillin (6 µg)	45 (100)
Oxacillin (5 µg)	45 (100)
Gentamicin (15µg)	2 (4.44)
Erythromycin (15UI)	30 (66.67)
Lincomycin (15 µg)	14 (31.11)
Tétracyclin (30UI)	32 (71.11)
Ofloxacin (5 µg)	24 (53.33)
Trimethoprim-sulfamethoxazole (1,25+23,75 µg)	31 (68.89)

Table 3. Frequency of multidrug resistance in MRSA

	Resistance to four or more antibiotics					Total
Number of antibiotics	4	5	6	7	8	16
Number of isolates resistant	3	1	2	8	2	

commendable to assess the susceptibility of multidrug resistant strains to vancomycin, which seems to represent the only antimicrobial alternative although vancomycin is very expensive and not largely available in our community.

We did not confirm methicillin resistance by minimal inhibitory concentration techniques or perform molecular confirmation (*mec*A, *fem*A) of our MRSA strains because of budgetary limitations. However, in this study, we established baseline information of nasal carriage of *S. aureus* in a potentially ill Malagasy community and confirmed the importance of prior hospitalization and antimicrobial use as risk factors of *S. aureus* carriage.

Conclusion

Future Malagasy studies should address *S. aureus* colonization in the healthy population versus a hospitalized population and should identify the country's specific risk factors to develop preventive activities against MRSA spread. Collaborative studies with other medical centers would help define the extent of the problem. However, the results of this study showing a high rate of MRSA nasal carriage and resistance to other drugs indicate the need to establish a national strategic policy to decrease the spread of resistant *S. aureus*.

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