## ORIGINAL ARTICLE

AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY SEPTEMBER 2016 ISBN 1595-689X VOL17 No.4
AJCEM/1637

AFR. J. CLN. EXPER. MICROBIOL. 17 (4): 250-255 http://dx.doi.org/10.4314/ajcem.v17i4.5

# EVALUATION OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* NASAL CARRIAGE IN MALAGASY VETERINARY STUDENTS

**Rasamiravaka, T[1*], Nirinarimanana, A J[1], Rasamindrakotroka, A[1]**

**[1]Laboratory of Training and Research in Medical Biology, Department of Medical Biology, Faculty of Medicine, University of Antananarivo, Madagascar; [1]Laboratoire de Formation et de Recherche en BiologieMédicale, Département de BiologieMédicale, Faculté de Médecine, Universitéd'Antananarivo, Madagascar**

**Correspondence:* **Laboratoire de Formation et de Recherche en Biologie Médicale (LBM) 7 Rue Joel RAKOTOMALALA –Lot II H 11 Bis Faravohitra, Antananarivo Madagascar. Telephone: +261 32 61 903 38. E-mail: travaka@yahoo.fr**

**RUNNING TITLE: MRSA NASAL CARRIAGE INMALAGASY VETERINARY STUDENTS**

## ABSTRACT

**Purpose:** Populations that are frequently in contact with animals such as veterinary students have been demonstrated to be at risk of MRSA carriage.Thus, it is relevant to generate baseline data in MRSA nasal carriage and multidrug resistance among Malagasy veterinary students (Madagascar).**Method:**A cross-sectional study was carried out among veterinary students coming for laboratory training. After their wise consent, nasal swabs of the anterior nares were carried out; and *S. aureus*was isolated by selective chromogenic culture. They were then assessed for antimicrobial susceptibility.
**Results:** Nasal swabs of 155 Malagasy veterinary students (Sex-ratio M/F: 0.91), enabled to isolate 30 (19, 35%) *S. aureus*strains, among which 14 (46, 66 %) were méthicillin-resistant (MRSA). Risk factors analysis revealed that history of hospitalization, recent antibiotic intake and frequent contact with animals and livestock workers/veterinarians increase the risk of MRSA nasal carriage. Among MRSA nasal isolates, a high rate of multidrug resistance and particularly an intriguing resistance to gentamycin (20%) and vancomycin (7.14%) were observed.
**Conclusion:** These results suggest that MRSA is spreading in Malagasy community requiringa strategic policy againstmultidrug resistant strains.

**Key-words:** Madagascar, MRSA, Risk factors, Veterinary

# EVALUATION DU PORTAGE NASAL DE *STAPHYLOCOCCUS AUREUS* RESISTANT A LA METHICILLINE CHEZ LES ETUDIANTS VETERINAIRES MALGACHES

**Résumé: Objectifs:** Les populations qui sont souvent en contact avec des animaux telles que les étudiants vétérinaires ont été démontrées comme étant à risque de portage de *Staphylococcus aureus* résistant à la méticilline(SARM). Ainsi, il est pertinent de générer des données de référence dans le portage nasal SARM chez les étudiants vétérinaires malgaches (Madagascar).**Méthodes:**Une étude transversale a été menée auprès des étudiants en médecine vétérinaire. Après leur consentement éclairé, des écouvillons nasaux des narines antérieures ont été réalisées et ayant permisd'isolerdes *S. aureus* sur milieu culturechromogènique sélectif et la réalisation de tests de sensibilité aux antimicrobiens.
**Résultats:** Les écouvillons nasaux de 155 étudiants malgaches vétérinaires (sex-ratio H / F: 0,91), a permis d'isoler 30 (19, 35%) souches de *S. aureus* parmi lesquels 14 (46, 66%) étaient résistantes à la méticilline (SARM). L'analyse des facteurs de risque a révélé que l'histoire de l'hospitalisation, la récente prise d'antibiotiques et les fréquents contacts avec les animaux et les travailleurs d'élevage/vétérinaires augmentent le risque de SARM portage nasal. Parmi les isolats de SARM, un taux élevé de multirésistance et en particulier une résistance intéressante à la gentamycine (20%) et à la vancomycine (7,14%) ont été observés.
**Conclusion:** Ces résultats suggèrent que le SARM se répand dans la communauté malgache nécessitant une politique stratégique contre les souches multirésistantes.

**Mots-clé:** Madagascar-MRSA-Facteurs de risque-Vétérinaire

## INTRODUCTION

*Staphylococcus aureus*and methicillin-resistant *S. aureus* (MRSA) are known as an invasive human pathogen responsible of serious infections in both hospitals and community. Moreover, it has been-established that nasal carriage of MRSA represents a major risk factor for subsequent infection and transmission of this pathogen [1, 2]. Likewise, several studies have confirmed asymptomatic colonization of animals at veterinary clinics worldwide and veterinarian has been identified as high-risk group for asymptomatic MRSA carriage, likely because of their close animal contact [3]. However, MRSA nasal carriage rate vary widely among countries. For instance, prevalence of 4.6% MRSA nasal carriage was documented among Dutch veterinary doctors and students with history of contact with livestock [4]. MRSA carriage was 12.3% in UK veterinarians attending MRSA-infected animals [5]. An international study reported that 12.5% (34/272) of veterinarians from nine country carried MRSA in the nares or their throat[6]. In a study of Brazilian university students, the percentage nasal carriage of *Staphylococcus aureus*was 40.8% with 5.8% of MRSA strains where all of them were susceptible to most of the antimicrobial agents tested [7].

Although, there is a worldwide increase in the number of infections caused by MRSA, the Pasteur institute of Madagascar reported that the prevalence of MRSA in *S. aureus* infection in Malagasy community still very low (5.8 %)[8]. In parallel, cross-sectional studies of unexposed Malagasy community reported 38.16 % of *S. aureus*nasal carriage with 14.8 % of MRSA strains[9]. However, to the best of our knowledge, no data concerning the frequency of MRSA nasal carriage among potentially exposed Malagasy community is available yet. Determining the prevalence of nasal carriage among exposed population is important in public health in so far as it strongly contributes to the update of the susceptibility of *S. aureus*to various drugs largely used in our community. Veterinarian students are considered as exposed population due to frequent contact with animal during their training. Thus, we assessed the *S .aureus*nasal carriage state of the veterinarian students coming for training in the Laboratory of Training and Research in Medical Biology of Madagascar, in order toestimate MRSA colonization in particular group of Malagasy community which is progressively in contact with domesticated animals and to identify some colonization risk factors.

## MATERIALS AND METHODS

### Sampling procedures

After a wise consent of veterinarian students coming for lab training, nasal swabs of the anterior nares were carried out by qualified technician according to the French C-CLIN (Centrede Coordination de la Luttecontre les Infections Nosocomiales) recommendation procedure[10]. Voluntary participants in the five academic level years (University of Antananarivo, Madagascar) completed anonymously, a very brief questionnaire designed to identify status and potential risk factors for staphylococcal colonization, including age, gender, previous hospitalization and antimicrobial use, frequent contact with animals or healthcare workers or veterinarians/livestock workers. Approval of the appropriate ethical committee had been obtained

### Bacterial identification and antimicrobial susceptibility

Single swab from each volunteer was immediately inoculated in Columbia blood Agar 5 % and incubated for 24h at 37°C. Plates were read at 24 h and *Staphylococcus aureus* isolates were identified according to their colony morphology, Gram-positive stain, positive catalase reaction, positive tube coagulase assay and Slidex Staph Kit ® (Biomerieux ®, France). Then *Staphylococcus aureus* isolates were inoculated onto selective chromogenic MRSA agar supplemented with 4 µg/mL of cefoxitin from CONLAB® for isolation of methicillin resistant *S. aureus*. Methicillin resistance *S. aureus* was confirmed by demonstration of blue colony growth on selective chromogenic MRSA agar [11]. Finally, susceptibility of MRSA to eight antibiotics (oxacillin, penicillin, erythromycin, vancomycin, ciprofloxacin, tetracycline, trimethoprim-sulfamethoxazole, gentamicin, clavulanicacid+amoxicillin) was assessed by disc diffusion technique following the guidelines of the Antibiogram Committee of French Society for Microbiology (CASFM) [12]. Briefly, an inoculum of $10^6$ CFU/ml was prepared and seeded in a Mueller-Hinton square plate. After an incubation of 24h at 37°C, the inhibition zone around antibiotic disks (Biorad®) was measured. For susceptibility to oxacillin, an inoculum of $10^7$ CFU/ml was prepared and the plate was incubated at 37°C for 24 hours on Mueller-Hinton agar + 2% NaCl. The breakpoints for resistance were those recommended by the CASFM [12]. Reference *S. aureus* ATCC® 25923 strains have been used as a quality control.

Multidrug resistance was defined as resistance to penicillin and oxacillin plus two or more antibiotics listed previously.

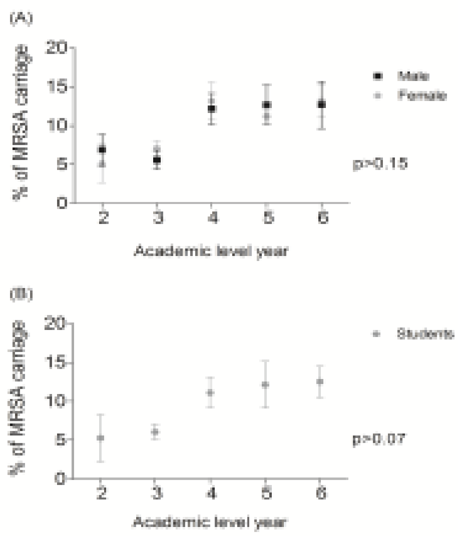
### Statistical analysis

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 association between 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 by using the Graphpad prism5® software.

## RESULTS

In two months, our lab received 210 veterinarian students for training during academic year 2014. Finally, nasal swabs were collected from 155 (73.80%) consenting students, sex-ratio M/F: 0.91with a mean ± SD age of 23 ± 5.55 years. *S. aureus* was isolated from 30 of 155 (19.35%) of students (Table 1).

**Figure 1: Frequency of MRSA colonization depending on gender and academic level year. A Frequency of MRSA colonization according to gender; B Frequency of MRSA Colonization According To Academic Level Year.**




Sixteen (53.34%) individuals were colonized with MSSA and fourteen (46.66%) were colonized with MRSA, for an overall estimate of MRSA colonization prevalence of 9.04%.

There was no significant association between sex and MRSA colonization (Fig A, B) nor between academic level year and MRSA colonization (P=0.06, P=0.75).

Recent antibiotic use (within one month)and history of hospitalization (OR, 5.13, 95% CI, 1.64–16.03; P=0.002; OR, 5.38, 95% CI, 1.72–16.77; P=0.002, respectively) were identified as being associated with MRSA colonization. Moreover, frequent contact with animals and livestock workers/ veterinarians (OR 5.28, 95% CI 1.14 to 24.47; P=0.02; OR 3.70, 95% CI 1.20 to 11.37; P=0.02, respectively) were identified as being associated with MRSA colonization. Most of the MRSA strains (14/30) expressed heterogeneous character according to the present of isolated colony close to the oxacillin disc inhibition zone. All MSSA strains were resistant to penicillin and resistance rate of MRSA to the other antibiotics tested are shown in Table 2.TeenMRSA strains were multidrug resistant among which eight (26.66%) MRSA isolates were resistant for eight antibiotics except vancomycin while three (10%) strains were resistant for all antibiotics.

## DISCUSSION

This present study is the first document of the prevalence of *S. aureus*and MRSA nasal colonization among veterinary students in Malagasy community. Our result, 9.04 % (n=14)of MRSA nasal carriage is higher than those documented in Netherland and Brazil afore mentioned [4, 7]. Likewise, MRSA nasal carriage of Danish veterinary practitioners (means professionally exposed to animals) was 3.9% [13] which is similar to the MRSA carriage rate in the general population (healthy individuals and outside the healthcare environment) estimated to be less than 4 %[14-15].However, this recorded rate seems to be lower compared to those reported among veterinarians from international study (12.5%)[6], from UK veterinarians (12.3%)[5] and intriguingly among unexposed Malagasy population (14.8%)[9]. In one hand, a lower rate could be comprehensive as students are considered as healthy population, with limited risk till they do frequently in touch with high risk factor area such as health and veterinarian care unit. In the other hand, a progressive increase of nasal carriage rate is predictable as students are progressively exposed to health-care facility without an effective hospitalization which may contribute to explain this intermediate rate. However, our results show no significant nasal carriage risk according to academic level year. Likewise, occidental studies yield a predominance of sex male in *S. aureus* carriers [16-17]. Herein, lack of association between *S. aureus* carriage and gender may be attributed to our lack of male (sex-ratio M/F: 0.91).However, it may suggest that there is no host influence at all. In that, influence of host habits and environment like hygiene habits may be more interesting than the only host status and should be well-explored.

It's particularly difficult to suppose a community-acquired MRSA (CA-MRSA) nasal carriage for those carriers even with lack of hospitalization history (n=119). Indeed, all of them, except the first academic year are frequently in contact with healthcare unit (medical lab and veterinary lab). Moreover, we didn't ask for an eventual ambulatory hospitalization or a recurrent hospital visits (as a visitor) which could represent risk factors for hospital-acquired MRSA colonization. Likewise, due to the impossibility to know the hospitalization history of MRSA nasal carriers, we couldn't identify a nosocomial nasal carriage. Those points are desirable in the way to distinguish the susceptibility of CA-MRSA strains to hospital-acquired one. One can assume that all multidrug resistant MRSA nasal carriers (n=16) presented are prior to hospitalization.

TABLE 1: CHARACTERISTICS OF STUDENTS COLONIZED BY *STAPHYLOCOCCUS AUREUS*AND METHICILLIN-RESISTANT *S. AUREUS* (MRSA)

| Characteristic | All participants (n=155) | With *S. aureus* result test n (%) | | | With MRSA result test n (%) | | |
|---|---|---|---|---|---|---|---|
| | | Negative=125 | Positive =30 | *p* | Negative =141 | Positive =14 | *p* |
| Age, mean ± SD, years | 23.47 ± 7.55 | 22.5 ± 6.5 | 24.75 ± 8.75 | | 25 ± 6.5 | 26 ± 7.2 | |
| Male | 74 (47.74) | 58(46.40) | 16 (53.33) | | 64 (45.39) | 10 (71.42) | |
| Female | 81 (52.26) | 67(53.60) | 14 (46.67) | | 77 (54.61) | 4 (28.58) | |
| Previous[a]antimicrobial use | 30 (19.35) | 16 (53.33) | 14 (46.67) | | 23 (76.66) | 7 (23.34) | .002 |
| Previoushospitalization | 36 (23.22) | 17 (42.22) | 19 (47.78) | | 28 (77.77) | 8 (22.23) | .001 |
| Frequent contact with healthcare workers[b] | 36 (23.22) | 19 (47.78) | 17 (42.22) | | 33 (91.66) | 3 (8.34) | |
| Frequent contact withanimals[b] | 87(56.13) | 40(45.97) | 47(54.03) | | 75 (86.20) | 12(13.80) | .02 |
| Frequent contact with animal workers[b] | 37(23.87) | 19(51.35) | 18(48.65) | | 30 (81.08) | 7(18.92) | .02 |

[a] within one months; [b] more than once a week

TABLE 2: ANTIBIOTIC RESISTANCE PROFILES OF 30 *S. AUREUS* (SA) AND 14 MRSA NASAL ISOLATES AS DETERMINED BY DISK DIFFUSION

| Antibiotics | SA (n=30) | MRSA(n=14) |
|---|---|---|
| | No (%) | No (%) |
| Penicillin | 30 (100) | 14 (100) |
| Oxacillin | 00 (100) | 14 (100) |
| Gentamycin | 06 (20) | 06 (42.85) |
| Erythromycin | 18 (60) | 09 (64.28) |
| AMC | 16 (53.33) | 06 (42.85) |
| Tétracyclin | 21 (70) | 11 (78.57) |
| Ofloxacin | 18 (60) | 08 (57.14) |
| Trimethoprim-sulfamethoxazole | 23 (76.67) | 10 (71.42) |
| Vancomycin | 01 (07.14) | 01 (07.14) |

In comparison with MRSA isolated from potentially ill Malagasy community reported in our previous study[9], MRSA nasal strains from veterinary students present similar rates of resistance, particularly for trimethoprim-sulfamethoxazole (68.89 % versus 71.42 %),erythromycin (66.67 % versus 64.28 %), tetracycline (71.11 % versus 78.57 %) and ofloxacin (53.33 % versus 57.14 %). Drug resistance of MRSA concern essentially the most antimicrobial used in our community. As a matter of fact, these antimicrobials are accessible to anyone without any medical prescription and very used even in non-bacterial infection. Intriguingly, resistances to gentamycin (20%) and vancomycin (7.14%) are unusual. As students are familiar to hospital and healthcare unit they can easily obtain hospital antibiotics compared to non-student one although we could not evidence real use of these antibiotics. However, these facts suggest the influence of antibiotics consumption habit in our population which can increase the acquisition of drug resistance by adaptive mutation [18].

In our study, identified risk factors for MRSA colonization were consistent with those yielded in other studies [16, 19]. Indeed, history of antibiotics use and hospitalization as well as frequent contact with animals and livestock workers/veterinariansincreases MRSA colonization (Table 1).Although, we couldn't establish a molecular characterization (*mec*A, *fem*A) of our MRSA strains to confirm MRSA identification, we establish baseline information of nasal carriage of MRSA in Malagasy veterinary student and confirm the place of prior hospitalization and antimicrobial use as high-risk factors of MRSA carriage. However, our samples are represented by a restricted population that comes to a particular health-care facility so that any extrapolation is hazardous. Furthermore, we were not able to distinguish neither their locality origin (townsman or peasant), nor their activities and social conditions that may have an influence in the carriage rate.

In our country, future studies should be addressed to *S. aureus* colonization in healthy population and really inpatients ones. Identification of specific risk factors is strategically important for preventive activities against MRSA spread. In this aspect, systematic screening at hospital admission should be debated as well as improved individual hygiene, decontamination of colonized individuals [20].Besides, longitudinal studies should be led to evaluate the progression of carriers and non-carriers through years of university study. Such studies could address the matter of persistent and intermittent carriers. Finally, identification of relevant pets contact and screening of MRSA colonization in frequently cared animals should contribute to evaluate reservoirs and transmission origins. However, the presence of high rate of MRSA nasal carriage and the increase of their resistance to other drugs in our community are disquieting. Without waiting for a nationwide survey results, it's highly recommended to establish a strategic policy in order to slow down the spread of these strains by different preventive measure such as control of antibiotic use.

**Acknowledgments:** None

**Source of support:** None

**Conflicts of interest:** None

**REFERENCES**

1. Wertheim HF, Melles DC, Vos MC, van Leeuwen W, van Belkum A, Verbrugh HA, et al,The role of nasal carriage in *Staphylococcus aureus*infections, Lancet Infect Dis, 2005; 5:751-62.
2. Wertheim HF, Vos MC, Ott A, van Belkum A, Voss A, Kluytmans JA, et al,Risk and outcome of nosocomial *Staphylococcus aureus*bacteraemia in nasal carriers versus non-carriers, Lancet, 2004; 364: 703-5.
3. Murtaza M, Malehah MN, Jawad SM, Saima S. Methicillin–Resistant *Staphylococcus aureus* as a Zoonotic Pathogen: How Safe is to Pat Kitty? IJRANSS, 2013;1(7): 1-10.
4. Wulf M, van Nes A, Eikelenboom-Boskamp A, de Vries J, Melchers W, Klaassen C, et al, Methicillin-resistant *Staphylococcus aureus*in veterinary doctors and students, the Netherlands, Emerg Infect Dis, 2006; 12:1939-41.
5. Loeffler A, Pfeiffer DU, Lloyd DH, Smith H, Soares-Magalhaes R, Lindsay JA.Meticillin-resistant *Staphylococcus aureus* carriage in UK veterinary staff and owners of infected pets: new risk groups, J Hosp Infect, 2010; 74(3):282-8.
6. Wulf M, Sorum WH, van Nes A, Skov R, Melchers W, Klaassen C, et al, Prevalence of methicillin resistant *Staphylococcus aureus* among veterinarians: an international study, ClinMicrobiol Infect, 2008; 14:29-34.
7. Prates KA, Torres AM, Garcia LB, Ogatta SF, Cardoso CL,Tognim MC.Nasal carriage of methicillin-resistant *Staphylococcus aureus* in university students,Braz J Infect Dis, 2010; 14(3):316-8.
8. Randrianirina F, Soares J-L, Ratsima E, Carod J-F, Combe P, Grosjean P, et al, In vitro activities of 18 antimicrobial agents against *Staphylococcus aureus* isolates from the Institut Pasteur of Madagascar, Ann ClinMicrobiolAntimicrob, 2007; 6, 5. doi:10.1186/1476-0711-6-5.
9. Rasamiravaka T, Rasoanandrasana S, Razafindraibe NJ, RakotoAlson AO, Rasamindrakotroka A. Evaluation of methicillin-resistant *Staphylococcus aureus* nasal carriage in Malagasy patients, *J* Infect in DevCtries,2013; 7(4):318-22.
10. Baron R, Borgey F, Chabaud-Mayer M, Chedeville E, Delhomme J, Delille F, et al, Coordination center of the fight against nosocomial infections western inter region. Detection of MRSA carrier; recommandations note 2007.
11. Hutchison MJ, Edwards GFS, Morrison D. Evaluation of chromogenic MRSA Reference Laboratory presented at the 2005 Institute of Bio Medical.
12. Skov R, Smyth R, Larsen AR, Bolmstrom A, Karlsson A, Mills K, et al,CASFM (Antibiogram Committee of French society for Microbiology): Recommandations 2012. Paris, France.
13. Moodley A, Nightingale EC, Stegger M, Nielsen SS, Skov RL, Guardabassi L. High risk for nasal carriage of methicillin-resistant *Staphylococcus aureus* among Danish veterinary practitioners, Scand J Work Environ Health, 2008; 34(2):151-7.

14. Malik S, Vranken P, Silio M, Ratard R, Van Dyke R. Prevalence of community-associated methicillin-resistant *Staphylococcus aureus* colonization outside the healthcare environment, Epidemiol Infect, 2009;137: 1237–41.
15. Rim JY, Bacon AE. 3rd. Prevalence of community-acquired methicillin resistant *Staphylococcus aureus* colonization in a random sample of healthy individuals. Infect Control HospEpidemiol, 2007; 28: 1044-6.
16. Nouwen JL, van Belkum A, Verbrugh HA. Determinants of *Staphylococcus aureus*nasal carriage, Neth J Med, 2001;59:126-33.
17. Halablab MA, Hijazi SM, FawziMA,Araj GF. *Staphylococcus aureus* nasal carriage rate and associated risk factors in individuals in the community, Epidemiol Infect, 2010; 138(5):702-6.
18. Levin BR, Perrot V, Walker N. Compensatory mutations, antibiotic resistance and the population genetics of adaptive evolution in bacteria. Genetics, 2000; 154:985-97.
19. Graffunder EM, Venezai RA. Risk factors associated with nosocomial methicillin-resistant *Staphylococcus aureus* (MRSA) infection including previous use of antimicrobials, J AntimicrobChemother, 2002; 49(6): 999-1005.
20. Haley CC, Mittal D, Laviolette, A, Jannapureddy S, Parvez N, Haley RW. Methicillin-resistant *Staphylococcus aureus*infection or colonization present at hospital admission: multivariable risk factor screening to increase efficiency of surveillance culturing, J ClinMicrobiol, 2007; 45: 3031-8.