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Original Article

Evaluation of methicillin-resistant *Staphylococcus aureus* nasal carriage in Malagasy pig and poultry non-industrial farmers

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Abstract

Introduction: The laboratory of Training and Research in Medical Biology of Madagascar conducted a cross-sectional study to estimate the rate of *S. aureus* nasal carriage of pig and poultry Malagasy farmers.

Methodology: Pig and poultry farmers from capital town of Madagascar were selected for nasal swabs collection with information on potential risk factors for *S. aureus* colonization, including animal exposure.

Results: Nasal swabs from 180 farmers (M/F sex ratio: 0.74), enabled isolation after culture and biochemical identification, 69 (38.33%) *S. aureus* strains among which 45 (25%) were methicillin-resistant (MRSA). Risk factors analysis revealed that farming duration, number of animals, direct contact with poultry, and frequent contact with manure increased risk of *S. aureus* and MRSA nasal carriage. Likewise, farm practices that imply close contact with pigs such as food distribution and pigsty washing increased risk of *S. aureus* and MRSA nasal carriage among pig farmers. Among MRSA isolates, resistance rate to other antibiotics was similar to that of MRSA isolates from the non-farmer Malagasy population. However, gentamycin resistance was noticeably higher (32.5% versus 4.44%).

Conclusions: This study shows a high rate of *S. aureus* and MRSA nasal carriage with high rate of multidrug resistance among healthy people frequently in contact with animals. A strategic policy against the spread of multidrug-resistant strains is desirable in farms and veterinary areas.

Key words: farmers; Madagascar; MRSA; risk factor.

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Introduction

Staphylococcus aureus is an invasive human pathogen responsible for serious infections and is particularly efficient at developing resistance to antimicrobials. Since the emergence of methicillin-resistant *S. aureus* (MRSA) in the United Kingdom in 1961 [1], increasing rates of methicillin resistance among *S. aureus* strains, particularly community-associated strains, have been a cause for concern [2]. Several animals, particularly pigs and calves, have been documented with MRSA colonization strains [3], and transmission of resistant bacteria from animal to farmers, called livestock-associated MRSA (LA-MRSA), has been demonstrated [4,5]. Interestingly, the prevalence of LA-MRSA strains generally ranges from 20% to 63%, and close contact with animals has been identified as a nasal carriage risk factor for farmers/veterinary workers [6,7].

In Madagascar, according to the study of the Pasteur Institute of Madagascar, the prevalence of MRSA in *S. aureus* infection in the Malagasy community still very low (5.8%) [8]. However, prevalence of *S. aureus* nasal carriage has been estimated to be 33.7% (116/304) with MRSA nasal carriage around 14.7% (45/304) among potentially ill Malagasy people [9]. To explain this high prevalence rate, the authors suspected the implication of self-medication and misuse and abuse of antibiotics. Indeed, antibiotic consumption is a well-known nasal carriage risk factor [2], and such drugs are available without prescription in the Malagasy community.

In this sense, and considering the particularity of livestock practices in the Malagasy community where animals are closely and frequently in contact with farmers homes, a high rate of LA-MRSA carriage among the farming community is expected. However, to the best of our knowledge, no data concerning the nasal carriage of MRSA in farmers with associated risk factors are available yet in the Malagasy community. Such a database could contribute to decisional policy in public health by updating the empiric antibiotic practices and establishing appropriate epidemiological surveillance in the healthy population, particularly those in contact with domestic animals.

In the present study, we assessed the *S. aureus* nasal carriage state of Malagasy farmers in the Laboratory of Training and Research in Medical Biology of Madagascar, in order to estimate MRSA colonization and to identify some colonization risk factors in this particular group frequently in contact with domesticated animals.

Methodology

Sampling and questionnaire information

After informed consent was obtained from pig and poultry farmers, nasal swabs of the anterior nares were carried out by a qualified technician based on the recommended procedure of the French C-CLIN (Centre de Coordination de la Lutte contre les Infections Nosocomiales) [10]. A systematic random sampling method was chosen to get enough samples for baseline prevalence from four well-known pig and poultry farm localities (Ampanotokana, Andraisoro, Antsahameva, Tsarahonenana) in the capital town of Madagascar. All non-industrial farmers that agreed to nasal swabs were enrolled in this study. To avoid cross-risk factors, exclusion criteria included (i) industrial farmers, (ii) farmers who simultaneously breed both pigs and poultry, (iii) farmers with frequent contact with industrial livestock (and slaughterhouse) or industrial livestock workers, and (iv) farmers who had been hospitalized within the last six months.

Participants completed a very brief questionnaire designed to identify status and potential risk factors for staphylococcal colonization, including age, gender, previous hospitalization, antimicrobial use, number of pigs and poultry per farm, farming duration, and farm practices (food distribution, pigsty washing, contact with manure, and proximity to poultry).

MRSA identification and antimicrobial susceptibility

Collected single swabs were immediately inoculated in 5% Columbia blood agar (Oxoid SAS, Thermo Fischer Scientific, Dardilly, France) and incubated for 24 hours at 37°C. Plates were read at 24 hours and *Staphylococcus aureus* isolates were identified based on colony morphology, Gram-positive stain, positive catalase reaction, positive tube coagulase assay, and Slidex Staph Kit (bioMerieux, Craponne, France). Then *S. aureus* isolates were inoculated onto selective chromogenic MRSA agar supplemented with 4 µg/mL of cefoxitin (Conda-Pronadisa, Madrid, Spain) for isolation of MRSA. MRSA 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, ofloxacin, tetracycline, trimethoprim-sulfamethoxazole, gentamycin) was assessed using the disk 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 (Oxoid SAS, Thermo Fischer Scientific, Dardilly, France). After incubation for 24 hours at 37°C, the inhibition zone around antibiotic disks (Bio-Rad, Hercules, USA) 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 (Oxoid SAS, Thermo Fischer Scientific, Dardilly, France) have been used as a quality control. No molecular typing was done to identify livestock-associated MRSA such as ST-398. 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* and MRSA colonization. Categorical comparisons were performed using χ^2 analyses, and $p < 0.05$ was considered significant for all comparisons. Logistic regression including univariate and multivariate calculations was done using GraphPad Prism 5 and XLSTAT software, with *S. aureus* nasal carriage in farmers as the dependent variable. All binary determinants with univariate Chi-square p values of ≤ 0.20 were eligible for multivariate analysis.

Results

S. aureus and MRSA carriage in farmers

During two months in 2014, 221 farmers were solicited and 180 farmers were finally enrolled, meeting the inclusion criteria. Nasal swabs were collected from 90 pig farmers and 90 poultry farmers. The M/F sex ratio was 0.74 with a mean ± SD age of 23 ± 17. 55 years. *S. aureus* was isolated from 69 of 180 (38.33%) patients. In total, 24 (34.78%) individuals were colonized with MSSA and 45 (65.22%) were colonized with MRSA, for an overall estimate of MRSA colonization prevalence of 25% (22.22% and 27.78% for pig and poultry farmers, respectively).

There was no significant association neither between age and *S. aureus* colonization nor between sex and *S. aureus* colonization (See Tables 1 and 2 for odds ratios). Chance of *S. aureus* nasal carriage

significantly increased with farming duration (particularly > 5 years) and number of animals in contact with farmers (particularly > 8 pigs and > 20 poultry animals). In addition, contact with manure and proximity to animals (proximity was considered when the animals' house was very close to the farmer's house) were identified as being associated with *S. aureus* colonization for poultry farmers (OR = 3.731, 95% CI = 1.477–9.425; p = 0.004 and OR = 6.000, 95% CI = 2.302–15.640; p = 0.0002, respectively, Table 1). Moreover, people in charge of food distribution and pigsty washing had a higher chance of *S. aureus* nasal carriage (OR = 3.472, 95% CI = 1.341–8.988; p = 0.010 and OR = 2.824, 95% CI = 1.160–6.873; p = 0.021, respectively, Table 2). Intriguingly, antibiotic use, history of hospitalization, and contact with healthcare workers were not significant risk factor for *S. aureus* nasal carriage, although frequent contact with healthcare workers and a history of hospitalization tended to increase nasal carriage (See Tables 1 and 2 for odds ratios). As shown in Table 3, multivariate analysis revealed that only proximity determinant had a significant association with nasal carriage.

Susceptibility testing of MRSA isolates

Most of the MRSA strains (39/45) expressed heterogeneous character based on the presence of isolated colony close to the oxacillin disk inhibition zone. In total, 15 MRSA strains were multidrug resistant, among which 12 (80%) MRSA isolates were resistant to 7 antibiotics, but all strains were susceptible to vancomycin.

Table 1. Characteristics of poultry farmers colonized by *S. aureus*.

Characteristic	Participants (n = 90)	*S. aureus* test results of poultry farmers					
		Negative (n = 55)	Positive (n = 35)		Univariate analysis		
			MSSA (n = 10)	MRSA (n = 25)	OR[d]	95% CI	p
Age, mean ± SD, years	24.16 ± 16.61	23.72 ± 16.01	24.32 ± 15.61	24.62 ± 14.51	0.985	0.280–5.889	0.657
Gender							
Male	40	25	0	15	0.900	0.383–2.11	0.809
Female	50	30	10	10			
Previous antimicrobial use[a]							
Yes	21	13	2	6	0.9573	0.350–2.615	0.932
No	69	42	8	19			
Previous hospitalization							
Yes	15	7	2	6	2.03	0.663–6.220	0.251
No	75	48	8	19			
Contact with healthcare workers[b]							
Yes	24	12	5	7	1.870	0.7251–4.820	0.226
No	66	43	5	18			
Contact with manure							
Yes	50	24	10	16	**3.731**	**1.477–9.425**	**0.004**
No	40	31	0	9			
Proximity to poultry[c]							
Yes	30	10	5	15	**6.000**	**2.302–15.640**	**0.0002**
No	60	45	5	10			
Livestock duration (years)							
< 1	27	22	2	3			
1–3	29	21	2	6	1.600	0.452–5.663	0.466
3–5	17	7	3	7	**7.333**	**1.804–29.816**	**0.005**
> 5	17	5	3	9	**10.560**	**2.539–43.918**	**0.001**
Number of poultry animals							
5–10	24	20	2	2			
11–15	30	22	2	6	1.818	0.474–6.974	0.383
16–20	20	11	3	6	**4.091**	**1.020–16.403**	**0.047**
> 20	16	2	3	11	**35.000**	**5.617–218.106**	**< 0.001**

MSSA: methicillin-sensitive *S. aureus*; MRSA: methicillin-resistant *S. aureus;* [a] Prior 6 months; [b] More than once a week; [c] Proximity was considered when the animals' house was close to the farmer's home; [d] Logistic regression of *S. aureus* nasal carriage (including MSSA and MRSA).

Table 2. Characteristics of pig farmers colonized by *S. aureus*.

Characteristic	Participants (n = 90)	Pig farmers with *S. aureus* result test n					
		Negative (n = 57)	Positive (n = 33)		Univariate analysis		
			MSSA (n = 13)	MRSA (n = 20)	OR[d]	95% CI	p
Age, mean ± SD, years	23.76 ± 16.41	23.72 ± 15.01	24.32 ± 12.61	23.62 ± 14.31	0.714	0.297–4.070	0.863
Gender							
Male	37	25	3	9	0.731	0.303–1.766	0.486
Female	53	32	10	11			
Previous antimicrobial use[a]							
Yes	32	21	5	6	0.857	0.347–2.113	0.737
No	58	36	8	14			
Previous hospitalization							
Yes	18	10	5	3	1.504	0.5269–4.293	0.585
No	72	47	8	17			
Contact with healthcare workers[b]							
Yes	50	29	8	13	1.813	0.7524–4.367	0.196
No	40	28	5	7			
Food distribution							
Yes	52	27	10	15	**3.472**	**1.341–8.988**	**0.010**
No	38	30	3	5			
Pigsty washing							
Yes	35	17	8	10	**2.824**	**1.160–6.873**	**0.021**
No	55	40	5	10			
Livestock duration (years)							
< 1	29	24	3	2			
1–3	23	17	3	3	1.676	0.472–5.952	0.424
3–5	28	16	3	9	**6.286**	**1.598–24.727**	**0.009**
> 5	10	0	4	6	**10.560**	**2.539–43.918**	**0.001**
Number of pigs							
2	52	44	2	6			
3	11	7	2	2	3.143	0.744–13.279	0.119
4	11	3	3	5	**14.667**	**3.189–67.451**	**0.001**
5	8	2	4	2	**16.500**	**2.814–96.748**	**0.002**
8	8	1	2	5	**38.500**	**4.154–356.82**	**0.001**

MSSA: methicillin-sensitive *S. aureus*; MRSA: methicillin-resistant *S. aureus;* [a] Prior 6 months; [b] More than once a week; [c] Logistic regression of *S. aureus* nasal carriage (including MSSA and MRSA).

Table 3. Multivariate analysis of nasal carriage among pig and poultry farmers.

		Univariate analysis			Multivariate analysis		
		OR	95% CI	p	OR	95% CI	p
Pig farmers							
	Food distribution	**3.472**	**1.341–8.988**	**0.014**	2.625	0.759–9.088	0.128
	Pigsty washing	**2.824**	**1.160–6.875**	**0.022**	1.585	0.470–4.881	0.489
Poultry farmers							
	Contact with manure	**3.731**	**1.477–9.425**	**0.004**	1.476	0.440–4.953	0.528
	Proximity to poultry	**6.000**	**2.302–15.64**	**0.0002**	**4.667**	**1.376–15.823**	**0.013**

Table 4. Antibiotic resistance profiles of 45 methicillin-resistant *S. aureus* (MRSA) nasal isolates as determined by disk diffusion.

	Poultry farmers	Pig farmers	% Average	OR[a]	95% CI	p
Antibiotics	**MRSA (n = 25) n (%)**	**MRSA (n = 20) n (%)**	**n = 45**			
Penicillin	25 (100)	20 (100)	100			
Oxacillin	25 (100)	20 (100)	100			
Gentamycin	09 (36)	05 (25)	30.5	0.592	0.16–2.17	0.42
Erythromycin	13 (52)	15 (75)	63.5	2.769	0.76–9.97	0.11
Tetracycline	11 (44)	16 (80)	62	5.091	1.31–19.66	0.01
Ofloxacin	05 (20)	15 (75)	47.5	12.00	2.93–49.11	0.0002
Trimethoprim-sulfamethoxazole	12 (48)	20 (100)	74	44.280	2.41–812.6	0.0001
Vancomycin	0 (0)	0 (0)	0			

[a] Logistic regression of antibiotic resistance among MRSA nasal carriers.

Among MRSA strains, resistance to gentamycin was higher (but not significantly) for poultry farmers compared with pig farmers, with an average of 30.5% (Table 4). In contrast, resistance to ofloxacin, tetracycline, and trimethoprim-sulfamethoxazole was significantly higher among pig farmers.

Discussion

The present study is the first to document the prevalence of *S. aureus* and MRSA nasal colonization among people frequently in contact with animals in a Malagasy community. Although the prevalence of *S. aureus* colonization (38.33%) in this sample is similar to world population estimate, that of MRSA colonization (25%) is higher compared to that reported from the unexposed Malagasy community (14.80%) [9]. However, this rate seems to be similar to (if not lower than) the population who is frequently in contact with animals, particularly pigs. Indeed, in Belgium, 37.8% (48/127) of pig farmers were colonized with MRSA [13], and in the Netherlands, more than 20% of pig farmers showed MRSA nasal carriage [14]. Moreover, 45% of swine workers were colonized with MRSA in the United States, suggesting an elevated transmission of MRSA from pigs to farmers [15]. Few studies have reported on MRSA colonization of poultry or chicken farmers. In Malaysia, low *S. aureus* prevalence (1.4%) among chicken flocks and the poultry farmers has been reported [16], and authors estimated that MRSA is absent or present only in low numbers among Malaysian flocks. Likewise, low prevalence in poultry was also found by Kitai *et al.* [17] and Lee [18], possibly because of the limited use of antimicrobial drugs in these animals. Interestingly, our study reports an unusually high MRSA colonization in poultry farmers (27.78%); risk factors related to frequent contact with poultry manure and proximity with poultry were significant (Table 1). Several studies suggested that LA-MRSA strains may be transmitted to humans in the veterinary setting [19,20] only after short-term exposure to animals. In Malagasy non-industrial livestock, farmers generally live, sleep, and eat near their animals, which undoubtedly increases their exposure to MRSA. However, we did not evaluate MRSA nasal carriage in pigs and poultry to correlate and confirm transmission hypotheses. In addition to the risk related to frequent contact with animals, risk for exposure to MRSA carriage can be related to poor hygienic practices. However, we were not able to identify specific hygiene-related practices such as frequency of handwashing, showering, or doing laundry that were associated with a reduced risk of *S. aureus* or MRSA colonization. One can assume that in a developing country such as Madagascar, difficulty of water supply undoubtedly reduces adequate hygienic practices.

Frequent contact with healthcare unit, hospitalization, and antibiotic use are generally assumed as risk factors [21]. In the present study, history of hospitalization, contact with healthcare workers, and recent antibiotic use did not significantly increase the rate of *S. aureus* and MRSA nasal carriage. On one hand, the lack of significant association could be related to the small population number or to an informative bias. Indeed, definition of antibiotics may be not the same for investigators and farmers (due to the lack of education among the latter), which could generate misunderstanding. Additionally, duration of hospitalization and contact with healthcare workers were not communicated and subject to confusion. On the other hand, those isolated MRSA strains may be neither hospital nor community-associated strains but rather LA-MRSA strains. This hypothesis should be confirmed by molecular typing analysis.

In comparison with MRSA isolated from potentially ill Malagasy community reported in our previous study [9], MRSA nasal strains from farmers presented similar high rates of resistance, particularly to trimethoprim-sulfamethoxazole (68.89% versus 74%), erythromycin (66.67% versus 63.5%), and tetracycline (71.11% versus 62%). This increase of drug resistance concerns antimicrobials that are frequently used in our community. These antimicrobials are available to anyone without a medical prescription and are often used even in non-bacterial infections such as the flu. These facts suggest the influence of the habit of antibiotic consumption in our population, which can increase microbial drug resistance by adaptive mutation [22]. When comparing the MRSA colonization of poultry and pig farmers, the high resistance rate to ofloxacin among MRSA isolated from pig farmers was expected, as fluoroquinolone is largely used for the treatment of respiratory and alimentary tract infections in pigs and poultry [23]. However, a similar resistance rate should be observed among MRSA isolated from poultry farmers. One assumption is that the proportion of accumulated antibiotics is probably not equal between pigs and poultry. In contrast, aminoglycoside (gentamycin) is not common in poultry treatment. Indeed, the antimicrobial drugs used in the flocks include generally tylosin, amoxicillin, trimethoprim-sulfamethoxazole, lincomycin, tetracycline, and colistin [23,24]. However, non-industrial farmers do not consult veterinary practitioners, which can lead to the use of inappropriate (but available) or insufficient antibiotics. Fortunately, the antimicrobial alternative vancomycin remains effective against MRSA strains of both pig and poultry farmers. However, we must keep in mind that this lack of resistance is probably related to the fact that this molecule is very expensive and not largely commercially available in our community. Moreover, the presence of heterogeneous MRSA strains should warn us because they require a bacteriologist's vigilance. Otherwise, MRSA strains may be identified as susceptible without using selective chromogenic agar. This fact emphasizes the importance of laboratory activity in the control of the spread of MRSA strains.

In this study, we established baseline information of *S. aureus* nasal carriage in pig and poultry Malagasy farmers and confirmed frequent contact with animals as an important risk factor of *S. aureus* carriage. However, our samples are represented by a restricted population, so any extrapolation is hazardous. Moreover, no causal relationship with pigs and poultry can be evoked as they were not tested. Thus, we cannot affirm that pigs and poultry are also highly colonized or that these animals are the reservoir of a livestock-associated strain. Likewise, no molecular typing was done to evaluate if isolated *S. aureus* are effectively livestock-associated strains. In this regard, future studies should be addressed to *S. aureus* and MRSA colonization in a larger population of industrial and non-industrial farmers as well as veterinary practitioners. Such studies should be coupled with the evaluation of *S. aureus* and MRSA colonization among pigs and poultry with identification of specific risk factors in order to build preventive action against the spread of LA-MRSA. Besides, the molecular characterization of *S. aureus* and MRSA strains *fem*A, *mec*A, and C01 for sequence type 398, as well as detection of clonal dissemination and production of toxins such as Panton–Valentine leucocidin, should be addressed for confirmation and epidemiological aspects such as the emergence and spread of atypical clones.

Conclusions

The presence of a high rate of MRSA nasal carriage and the increase of their resistance to other drugs in our community are disquieting. Without waiting for nationwide survey results, establishing a strategic policy in order to slow down the spread of these strains by different preventive measures, such as control of antibiotic use, is highly recommended.

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Authors' contributions

ATT participated in the collection of strains and susceptibility testing. RT drafted the manuscript and performed the statistical analysis. RT conceived the study and participated in its design and coordination. All authors read and approved the final manuscript. RT and RA are guarantors of the paper

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