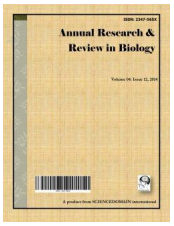

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Methicillin-resistant *Staphylococcus aureus* Nasal Carriage in Poultry Farmers and Poultry Slaughterers in Ouarzazate - Morocco

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Authors' contributions

This work was carried out in collaboration between both authors. Author AC designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author FRF managed the analyses of the study. Both authors read and approved the final manuscript.

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ABSTRACT

Aim: The aim of this study were to assess the prevalence of nasal carriage of *S. aureus* and MRSA among moroccan farmers and slaughterers of poultry, to determine the risk factors associated with this carriage and to evaluate susceptibility of isolated strains to antibiotics.
Methodology: Poultry farmers and slaughterers from Oarzazate-Morocco were selected for nasal swabs collection with information on potential risk factors for *S. aureus* colonization. Isolation, identification and antimicrobial susceptibility of this pathogen were performed according to the conventional methods of bacteriology.
Results: Nasal swabs from 52 poultry workers enabled isolation of 25 (48.07%) *S. aureus* strains among which 8 (15.38%) were methicillin-resistant (MRSA). Risk factors analysis revealed that seniority in the profession and livestock /slaughterhouse duration increased risk of *S. aureus* and

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MRSA nasal carriage. Isolated strains presented high rates of resistance to antibiotics, particularly to Penicillin, Tetracycline and Erythromycin. However, all of them were still susceptible to vancomycin.

Keywords: Poultry farmers; slaughterers; Morocco; MRSA; risk factor.

1. INTRODUCTION

Antibiotic resistance is a growing world-wide issue because of its effect on the rapid spread of threatening diseases and infections and the inability to control them. *Staphylococcus aureus* is one of the most common human pathogens. There is notorious for its ability to become resistant to antibiotics. The multidrug resistance and the nasal carriage of *S. aureus* play a key role in the epidemic of the infections [1,2]. Methicillin-resistant *S. aureus* (*MRSA*) is a bacterium that causes infections in different parts of the body. It's tougher to treat than most strains of *S. aureus* because it's resistant to some commonly used antibiotics.

Livestock are a well-known reservoir for this pathogen, which poses substantial health risks for livestock workers. Methicillin-resistant *S. aureus* (MRSA) strains have been isolated from pigs, cattle, and poultry in numerous European countries [3,4]. The colonized animals are a reservoir of MRSA not only for livestock but also for humans with close contact to animals, i.e., farmers, veterinarians and slaughterers. Higher colonisation rates and cases of infections have been reported in these professions at risk [5,6,7].

Data from surveys in the United States and Europe suggest that there is a risk of increased colonization by resistant bacteria in farm staff associated with exposure in the workplace [8]. 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 Moroccan community.

The purpose of this study is to assess the *S. aureus* nasal carriage state of moroccan poultry farmers and slaughterers, in order to estimate multidrug-resistant *S. aureus* (MDRSA) and methicillin-resistant *S. aureus* (MRSA) colonization and to identify some colonization risk factors in this particular group frequently in contact with poultry. The realization of such an investigation in Morocco is justified by the differences of practice that exist between countries in terms of antibiotic use in livestock.

2. MATERIALS AND METHODS

2.1 Sample Collection

Our study was carried out from December 2016 to February 2017 in Ouarzazate -Morocco. After having explained the research's aim to Poultry farmers and slaughterers chosen at random sampling, their final selection was based on their willingness to participate in our study. Only four people declined.

Participants completed a very brief questionnaire designed to identify status and potential risk factors for staphylococcal colonization, including profession, age, gender, seniority in profession, prior hospitalization and antimicrobial use. To avoid cross-risk factors, we have excluded people who had been hospitalized within the last six months and people who received antibiotic treatment the month prior to inclusion in the study. Finally, we enrolled a total of 52 participants with direct contact with poultry. Twenty five participants were poultry farmers and 27 were slaughterers. Nasal swabs were carried out, based on the recommended procedure of the French C-CLIN (Centre de Coordination de la Lutte contre les Infections Nosocomiales) [9].

2.2 Isolation of *S. aureus*

The isolation and identification of *S. aureus* were performed according to the conventional methods of bacteriology: Collected single swabs were immediately inoculated in Columbia nalidixic acid (CNA) agar and incubated for 24 hours at 37°C. Plates were read at 24 hours and *S. aureus* isolates were identified based on colonial morphology, Gram stain, catalase, and tube coagulase test.

2.3 Antibiogram

The isolates were tested for susceptibility to oxacillin (5 µg), penicillin (6 µg), erythromycin (15UI), vancomycin (30 µg), ofloxacin (5 µg), tetracycline (30UI), trimethoprim-sulfamethoxazole (1,25/23,75 µg) and gentamycin (15 µg). The antibiogram was

performed by the agar diffusion method following the guidelines of the Antibiogram Committee of the French Microbiology Society (CASFM, 2012 edition). Quality control was carried out through the *S. aureus* strain ATCC 25923 provided by the laboratory of the National Reference Center for Staphylococci (CNRS) in Lyon, France. Multidrug resistance was defined as resistance to penicillin and oxacillin plus two or more antibiotics listed previously.

For isolation of MRSA, *S. aureus* isolates were inoculated onto selective chromogenic MRSA agar supplemented with 4 µg/mL of cefoxitin (Conda-Pronadisa, Madrid, Spain). MRSA was confirmed by demonstration of blue colony growth on selective chromogenic MRSA agar [10].

3. RESULTS

As shown in Table 1, *S. aureus* was isolated from 25 of 52 (48.07%) workers. In total, 17 (68%) individuals were colonized with methicillin-susceptible *S. aureus (*MSSA) and 8 (32%) were colonized with MRSA, for an overall estimate of MRSA colonization prevalence of 15.38% (7.69% for both poultry farmers and poultry slaughterers).

Only two of the five variables tested were significantly associated with *S. aureus* nasal carriage (Table 1). Seniority in the profession and livestock /slaughterhouse duration were identified as being associated with *S. aureus* colonization for workers. Indeed, risk of *S. aureus* nasal carriage significantly increased with seniority and livestock /slaughterhouse duration. In contrast, profession, previous antibiotic use, and history of hospitalization, were not significant risk factor for *S. aureus* nasal carriage. However, profession and previous hospitalization, tended to increase *S. aureus* nasal carriage.

Our results showed that all *S. aureus* strains were susceptible to vancomycin. Resistance rate to all antibiotics tested was significantly higher for MRSA strains compared with MSSA strains (Table 2). All MRSA strains showed a multidrug-resistant profile, among which 1 (12.5%) MRSA isolates were resistant to 5 antibiotics, and 2 (25%) isolate were resistant to 4 antibiotics (Table 3).

4. DISCUSSION

S. aureus nasal carriage was higher (but not significantly) for poultry farmers compared with poultry slaughterers. This result is related to frequent contact with poultry manure and proximity with poultry. The prevalence of *S. aureus* colonization in this study (48.03%) is higher compared to that reported from malagasy poultry farmers (38.88%) [11] and from nigerian poultry farmers (35%) [12]. However, this result is lower than those obtained in a study conducted at the Ibn Rochd University Hospital Center, Casablanca, Morocco [1], who reported a prevalence of 53.2% and 70.9% from nasal swab of cases and controls respectively. An estimated 30% of humans are nasal carriers of *S. aureus* [13], though carriage rates vary with geographic location, seasonality, age and sex [14]. Moreover, The prevalence of MRSA colonization (15.38%) is similar to that (14.29) reported by Kwoji et al. [12]. This rate is lower compared to that found by Rasamiravaka [11]. The difference in these prevalence could be attributed to the management practices by farmers and slaughterers in the study areas, sample sizes, bacterial isolation and identification methods.

Nasal carriage plays an important role for the transmission, autoinfection, and cross infection in the community. Since the 1950s, several authors have demonstrated the role of *S. aureus* carriage in the acquisition of infection with S. aureus/MRSA [1,15].

Few studies have reported on MRSA colonization of poultry farmers and slaughterers. The present study shows that a frequent and long contact with poultry significantly increase the rate of *S. aureus* and MRSA nasal carriage. However history of hospitalization, recent antibiotic use, and profession are not associated with this risk. Although, other studies have shown that these variables increase this risk. [11,16]. These different results could be related to the differences of practice that exist between countries in terms of antibiotic use in livestock or to population number.

MRSA nasal strains from farmers and slaughterers presented high rates of resistance to other antibiotics, particularly to Penicillin (100%), Tetracycline (100%) and Erythromycin (50%). This increase of drug resistance concerns antimicrobials that are frequently used. Additionally, these strains were extremely multi-resistant, them were resistant at least to two antibiotics other than methicillin, but all of them were still susceptible to vancomycin. This lack of resistance is probably related to the fact that this molecule is very expensive and not largely commercially available in our community [11].

Table 1. Characteristics of poultry workers (Farmers and Slaughterers) colonized by *S. aureus*

Characteristic	Participants (n = 52)	*S. aureus* test results of poultry farmers and slaughters					
		Negative (n = 27)	Positive (n = 25)		Univariate analysis [b]		
			MSSA (n = 17)	MRSA (n = 8)	OR	95% CI	p
Profession							
Poultry farmers	25	11	10	4	1.85	0.61 - 5.57	
poultry slaughterers	27	16	7	4			$p \leq 0.30$
Previous hospitalization							
Yes	26	12	11	3	1.59	0.53 - 4.75	
No	26	15	6	5			$p \leq 0.50$
Previous antimicrobial use [a]							
Yes	25	13	8	4	0.99	0.33 - 2.94	$p \leq 0.90$
No	27	14	9	4			
Livestock /Slaughterhouses duration (years)							
≥ 2	18	5	8	5	4.77	1.37 -16.62	
< 2	34	22	9	3			$p \leq 0.02$
Seniority in the profession							
≥ 1 an	20	6	9	5	4.45	1.34-14.82	$p \leq 0.02$
< 1 an	32	21	8	3			

MSSA: methicillin-sensitive S. aureus; MRSA: methicillin-resistant S. aureus; [a] *Prior 6 months;*
[b] *Univariate analysis of S. aureus nasal carriage (including MSSA and MRSA)*

Table 2. Antibiotic resistance profiles of methicillin-susceptible *S. aureus* (MSSA) and methicillin-resistant *S. aureus* (MRSA) nasal isolates

Antibiotics	Number of strains (%)		p
	MRSA n = 8	MSSA n = 17	
Penicillin	8 (100)	12 (70.58)	$p \leq 0.05$
Gentamycin	2 (25)	0	$p \leq 0.05$
Erythromycin	4 (50)	0	$p \leq 0.01$
Tetracycline	8 (100)	5 (29.41)	$p \leq 0.001$
Trimethoprim-sulfamethoxazole	2 (25)	0	$p \leq 0.05$
Vancomycin	0	0	--

Table 3. Resistance profiles of methicillin-resistant *S. aureus* (MRSA) strains from nasal swabs

Resistance profiles	MRSA strains (%)
P Te G E Tsx	1 (12.5)
P Te G E	1 (12.5)
P Te E Tsx	1 (12.5)
P Te E	1 (12.5)
P Te	4 (50)

P: Penicillin G; G: Gentamycin; E: Erythromycin; Te: Tetracycline; Tsx: Trimethoprim – Sulfamethoxazol

The mechanism of resistance to methicillin in *S. aureus* is mainly due to the production of the modified protein PBP2a (penicillin-binding protein 2a) encoded by the mecA gene which is carried in the mobile genetic element SCCmec [1,17].

5. CONCLUSION

The high prevalence of nasal carriage of *S. aureus* and MRSA among moroccan farmers and slaughterers of poultry and the increase of their resistance to other drugs are disquieting. we recommend to establish a strategy in order to slow down the spread of these strains by different preventive measures, such as control of antibiotic use, training and education of workers to safety measurenments.

CONSENT

As per international standard or university standard, patient's written consent has been collected and preserved by the authors.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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