



Nasal Carriage and Antimicrobial Resistance of *Staphylococcus aureus* in Household Pets and Their Owners in Algeria

ARTICLE INFO

Article Type
Original Article

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How to cite this article

Djebbar A., Sebahia M., Bekara MEA., Kaufa H., Goutal B., Namoune R., Gribi K. Nasal Carriage and Antimicrobial Resistance of *Staphylococcus aureus* in Household Pets and Their Owners in Algeria. Infection Epidemiology and Microbiology. 2024;10(2): 79-88

Article History

Received: November 07, 2023
Accepted: April 23, 2024
Published: June 21, 2024

ABSTRACT

Background: This study aimed to investigate the prevalence and potential risk factors of *Staphylococcus aureus* nasal carriage in household pets (cats and dogs) and their owners in Chlef province in Algeria and to determine the isolates antibiotic resistance profiles.

Materials & Methods: *S. aureus* was isolated from nasal swabs, identified by culture on mannitol salt agar (MSA), and confirmed by polymerase chain reaction (PCR) amplification of the *nuc* gene. Methicillin-resistant *S. aureus* (MRSA) isolates were identified by their resistance to cefoxitin and PCR targeting the *mecA* gene. Panton-Valentine leukocidin (PVL) toxin genes were screened by PCR. Antimicrobial resistance was determined by disc diffusion method. The effect of risk factors on *S. aureus* nasal carriage was evaluated using a multivariable generalized linear model (GLM).

Findings: A total of 110 nasal swabs were collected: 29, 31, and 50 from dogs, cats, and their owners, respectively. The nasal carriage rate of *S. aureus* was 25% in household pets (22.6% in cats and 27.6% in dogs) and 22% in their owners.

MRSA isolates were recovered only from pets (6.6%); 25% of them were multidrug resistant (MDR). One MDR MRSA isolate was PVL-positive. The age of dogs was the only risk factor significantly associated with *S. aureus* nasal carriage.

Conclusion: The results revealed that nasal carriage of *S. aureus* in household pets was relatively high, raising concern about their potential risk to human health and stressing the importance of active surveillance of *S. aureus* carriage in pets.

Keywords: *Staphylococcus aureus*, Methicillin-resistant, Pets, Nasal cavity, Antibiotic resistance, Risk factors

CITATION LINKS

[1] Tong SY, et al. *Staphylococcus aureus*... [2] Pantosti A, Venditti M. What is MRSA... [3] Wertheim HF, et al. The role of nasal carriage ... [4] Bramble M. Potential role of pet... [5] Harrison EM, et al. A shared population of epidemic... [6] European Pet Food Industry Federation... [7] Petinaki E, Spiliopoulou I. Methicillin-resistant *Staphylococcus*... [8] Abdullahi IN, Zarazaga M, Nasal *Staphylococcus*... [9] Mairi A, et al. Distribution of toxinogenic methicillin... [10] Zhang K, et al. New quadriplex PCR assay for... [11] Ryffel C, et al. Sequence comparison of *mecA* genes... [12] Sambrook J. Molecular cloning: A laboratory... [13] Lina G, et al. Involvement of Panton-Valentine ... [14] Magiorakos AP, et al. Multidrug-resistant, extensively ... [15] Alsolami A, et al. Community-acquired methicillin-resistant... [16] Team RDC. R: A language and environment for... [17] Rahman MM, et al. Investigation of methicillin-resistant ... [18] Mustapha M. Phenotypic and... [19] Decline V. Profile of... [20] Boost MV. Prevalence of *Staphylococcus*... [21] Mehraj J, et al. Methicillin-sensitive and ... [22] Penna B, et al. Comparative genomics of MRSA ... [23] Abdel-Moein KA. Methicillin-resistant... [24] Bean DC, Wigmore SM. Carriage rate and antibiotic... [25] Wan MT, Heterogeneity and phylogenetic relationships of... [26] Fabri FV, et al. First report of oxacillin-susceptible ... [27] Myaing TT, et al. Antimicrobial resistant pattern of ... [28] Bierowiec K. Prevalence and risk... [29] Hanselman BA. Coagulase positive... [30] Elmoslemayn A, et al. Diversity and risk factors... [31] Van Duijkeren E. Transmission of ... [32] Rankin S, Panton-Valentine leukocidin (PVL)... [33] Leistner R, Skin infections due to Panton-Valentine ... [34] Del Giudice P. Skin infections caused by... [35] Hiramatsu K, et al. Genomic basis for...

Introduction

Staphylococcus aureus is a major pathogen capable of causing a wide variety of infections, ranging from mild to severe, in both humans and animals [1]. The epidemiology of *S. aureus* underwent a significant change in the 1960s, following the emergence of a new strain of Methicillin-resistant *S. aureus* (MRSA). Resistance of MRSA to methicillin is due to the acquisition of a *mecA* gene, which confers resistance to most β -lactam antibiotics. The *mecA* gene is carried by a mobile genetic element located on the chromosome, called staphylococcal cassette chromosome *mec* (SCC*mec*). Certain clones of MRSA, called healthcare-associated MRSA (HA-MRSA), are predominant in hospital settings around the world, whereas other clones called community-associated MRSA (CA-MRSA), which emerged in the 1990s, cause infections in healthy individuals with no history of recent hospitalization. CA-MRSA strains produce a two-component toxin called Panton-Valentine leukocidin (PVL) (*LukS-PV* and *LukF-PV*), which is an important virulence factor associated with severe infections [2].

Approximately 25% of humans carry *S. aureus* in their nares. It is well documented that nasal carriage of *S. aureus* is a major risk factor for infection by this organism in community and hospital settings, and that nasal carriers of *S. aureus* are more prone to develop *S. aureus* infection than non-carriers [3]. Several animal species, particularly companion animals such as dogs and cats, could be colonized and infected with *S. aureus* and therefore may contribute to the transmission of infections among individuals sharing the same household as well as in the community in general [4]. Indeed, MRSA strains isolated from companion animals and humans in the same household have been reported to be identical, indicating that MRSA could be transmitted from humans to

animals and vice versa [5]. This risk of transmission has been exacerbated further in recent years due to the substantial growth of the companion animal population in modern society [6].

Therefore, it is important to take seriously the risk that pets may pose to human health and to assess this risk by screening for nasal carriage of *S. aureus* in pets and identifying risk factors that may be associated with interspecies transmission. Knowledge of these factors is crucial for the implementation of preventive strategies to control and prevent the transmission of infections in the community as well as in hospital settings.

While numerous studies on the prevalence of nasal carriage of *S. aureus* and MRSA in companion animals and their owners have been carried out around the world [7,8], to the best of our knowledge, no such studies have been carried out in Algeria, except for a previous study [9].

Objectives: this study was conducted to estimate the prevalence of *S. aureus* and MRSA in the nasal cavity of cats, dogs, and their owners in Chlef province in Algeria, to analyse the antibiotic resistance patterns of the isolates, and to assess the influence of certain risk factors on their nasal carriage.

Materials and Methods

Study population: Owners and their pets (cats and dogs) were recruited from eight veterinary practices in Chlef province, Algeria, between February and April 2023.

A questionnaire was completed during sampling to collect general and medical data about pets and their owners. Participants were identified using random and anonymous numbers.

Sample collection: Nasal swab samples were taken from both nostrils of the participants, including the animals and their owners. Veterinarians conducted the swabbing procedure, and each swab was

carefully placed in a sterile tube containing Stuart transport medium (Himedia, India). The collected samples were then promptly transported to the laboratory for processing on the same day.

Isolation and identification of bacteria:

A pre-enrichment step was performed by adding 2 mL of nutrient broth (Liofilchem, Italy) to each tube containing a nasal swab, followed by incubation at 37 °C for 24 hrs. Then 25 µL of the pre-enrichment broth was streaked onto mannitol salt agar (MSA) medium (Merck, Germany) and incubated at 37 °C for 24 to 48 hrs.

Suspicious colonies showing the characteristics of *S. aureus*, according to their morphology and yellowish color due to mannitol fermentation, were selected and sub-cultured on nutrient agar (Liofilchem, Italy) and then incubated at 37 °C for 24 to 48 hours to obtain pure colonies. These colonies were analysed by standard microbiological and biochemical tests, including Gram coloration, catalase, and coagulase tests.

Molecular Identification: All Gram-positive cocci that were positive for catalase and coagulase were identified by PCR first as *S. aureus* using a pair of primers (Eurofins Genomics, USA) specific for the *S. aureus nuc* gene, which encodes the nuclease [10], and then as MRSA using primers specific for the *mecA* gene (Eurofins Genomics, USA), which confers resistance to methicillin [11]. The sequences of the primers used are given in Table 1.

Chromosomal DNA extraction was carried out by boiling method following the protocol of Sambrook and colleagues (2001) [12]. Briefly, two colonies of *S. aureus* were re-suspended in 500 µL of sterile distilled water, homogenized by vortexing, incubated at 100 °C for 10 min, and then immediately placed on ice for 10 min. The suspension was centrifuged at 13,000 rpm for 10 min,

and the supernatant containing DNA was retained to serve as PCR template.

The amplification reactions were carried out in a total volume of 25 µL, containing 5 µL of DNA, 0.25 µL of each primer (50 µM), 4 µL of magnesium chloride (25 mM) (Promega, USA), 0.5 µL of mixture of dNTPs (25 mM) (Promega, USA), 2.5 µL of buffer (10X) (Promega, USA), and 0.25 µL of Taq polymerase (Promega, USA). The reaction mixture volume completed to 25 µL by adding 12.25 µL of free nuclease water. The thermocycler (Prime Techne; United Kingdom) PCR program used for each primer pair is shown in Table 1. PCR products were subjected to electrophoresis on 1.5% agarose gel containing ethidium bromide and visualized under a UV transilluminator (Daihan, Korea).

DNA from *S. aureus* strain ATCC® 25923 was used as a positive control for the detection of *nuc* and *pvl* genes, whereas an in-house strain was used as a positive control for the *mecA* gene.

Detection of the PVL genes: The presence of genes encoding two-component PVL toxin (*lukF-PV* and *lukS-PV*) was screened by PCR using a pair of primers *luk-PV-1* and *luk-PV-2* [13]. The primer sequences and the amplification program are given in Table 1.

Antibiotic sensitivity testing: *S. aureus* isolates were tested for their sensitivity to six antibiotics (Liofilchem, Italy), including erythromycin (E, 15 µg), clindamycin (DA, 2 µg), fusidic acid (FA, 10 µg), ciprofloxacin (CIP, 5 µg), ceftiofloxacin (FOX, 30 µg), and kanamycin (K, 30 µg), using disc diffusion method on Mueller-Hinton agar medium. The results were interpreted following the recommendations of the Antibiogram Committee of the French Society of Microbiology (CA-SFM) (https://www.sfm-microbiologie.org/CASFM2023_V1.0.pdf) and the CA-SFM guidelines VET2021 (<https://www.sfm-microbiologie.org/>)

Table 1) Primers and PCR conditions used for the detection of the *nuc*, *mecA*, and Panton-Valentine toxin genes

PCR Target	Reference	Primer	PCR Conditions	Product size
<i>nuc</i> gene	[10]	<i>Nuc</i> F: GCGATTGATGGTGATACGGTT <i>Nuc</i> R: AGCCAAGCCTTGACGAACTAAAGC	1 cycle: 94°C 3 min	279 bp
			35 cycles: 94°C 30 s 57°C 30 s 72°C 30 s	
<i>mecA</i> gene	[11]	<i>MecA</i> F: GTAGAAATGACTGAACGTCCGATAA <i>MecA</i> R: CCAATTCCACATTGTTTCGGTCTAA	1 cycle: 72°C 5 min	310 bp
			1 cycle: 94°C 3 min	
PVL toxin genes	[13]	<i>luk-PV-1</i> : ATCATTAGGTAAAATGTCTGGACATGATCCA <i>luk-PV-2</i> : GCATCAASTGTATTGGATAGCAAAGC	1 cycle: 72°C 5 min	433 bp
			1 cycle: 94°C /3 min	
			35 cycles: 94°C 30 s 55°C 30 s 72°C 30 s	
			1 cycle: 72°C 5 min	

casfm-veterinaire-2021/) for animals. MDR is defined as resistance to antibiotics belonging to three or more antibiotic classes [14, 15].

Statistical analysis: Data were analysed using R software (Version 4.1.1) [16]. Descriptive statistical analysis was used to calculate the prevalence of *S. aureus* and MRSA in household pets and their owners with 95% confidence interval (95% CI). In this analysis, categorical variables were presented as absolute and percentage frequencies.

Risk factors for *S. aureus* nasal carriage in cats, dogs, and their owners were identified using a multivariable generalized linear model (GLM). The selection of the final model was carried out in two steps. (i) Univariate analysis was performed using the likelihood ratio test. Variables with a *p*-value < .25 in the univariate analysis at 95% CI level were retained for step 2. (ii) A forward-stepwise variable selection process (starting with an empty model) using the Akaike information criterion (AIC) was used to select the best

model. The model with the lowest AIC value was chosen. All second-order interactions between covariates were tested in the final model. The selected variables in the final model with a *p*-value less than .05 were considered as significant.

Findings

A total of 110 nasal swabs were collected in this study: 60 from pets (29 from dogs and 31 from cats) and 50 from their owners. Among them, 55.3% (n=16) of dogs, 64.5% (n=20) of cats, and 64% (n=32) of their owners were male.

The age of the companion animals in this study ranged from 2 months to 17 years for cats and from 2 months to 8 years for dogs, and the majority of them were ≤ 2 years: 83.7% (n=26) of cats and 79.3% (n=23) of dogs. The age of the owners ranged from 13 to 58 years, and 54% (n=27) of them were over 25 years old.

Prevalence of *S. aureus* and MRSA: The nasal carriage rate of *S. aureus* was 22% (n=11) in pet owners (95% CI: 11-33%) and

25% (n=15) in pets (95% CI: 14-36%), with 25% (n=6 of 24) (95% CI: 8-42%) in dog owners, 19.2% (n=5 of 26) (95% CI: 4.1-34.3%) in cat owners, 27.6% (n=8) in dogs (95% CI: 11.4-43.8%), and 22.6% (n=7) in cats (95% CI: 7-38.2%). Of the 26 *S. aureus* isolates identified in this study, four (15.4%) isolates were resistant to ceftiofur and carried the *mecA* gene and therefore were considered as MRSA. These MRSA isolates were all isolated from pets, and none of the owners were positive for MRSA. The overall MRSA carriage rate was 6.6% (4 of 60) among pets (95% CI: 0.1-7.2%): 9.6% (3 of 31) in cats (95% CI: 0.8-20%) and 3.4% (1 of 29) in dogs (95% CI: 0-6.8%). MRSA accounted for 26.6% (4 of 15) of all *S. aureus* isolates from pets.

Detection of the PVL toxin genes: Among all *S. aureus* isolates obtained in the present study, one MRSA isolate (4%, 1 of 26) from a cat was positive for PVL toxin genes (*lukS-PV* and *lukF-PV*) by PCR.

Antimicrobial Resistance: The antibiotic resistance profiles of *S. aureus* isolates from pets and their owners are presented in Table 2. All the antibiotics tested were active against methicillin-susceptible *S. aureus* (MSSA) isolates from pets, whereas Fox, FA, and K antibiotics were the most active antibiotics against human *S. aureus* isolates (0% resistance). The resistance rate of all the isolates in this study ranged from 0 to 15.4%: from 0 to 26.6% in those isolated from household pets and from 0 to 18% in those isolated from their owners (Figure 1). Only one isolate, an MRSA isolate from a cat, was MDR and carried PVL-encoding genes.

Risk factors for nasal carriage: The risk factors for nasal carriage of *S. aureus* in household pets and their owners in Chlef province, Algeria are given in Table 3. The results of the multivariate model showed that among all the risk factors analysed in this study, only the age in dogs was statistically

Table 2) Antibiotic resistance pattern of *Staphylococcus aureus* isolates from pets and their owners

Isolate	Antibiotic ($\mu\text{g/mL}$)					
	FOX (30)	K (30)	E (15)	CIP (5)	DA (2)	FA (10)
From cats						
02 EXT	S	S	S	S	S	S
08 EXT	S	S	S	S	S	S
02 BENF	S	S	S	S	S	S
08 BENS	S	S	S	S	S	S
09 BENS	R	S	R	S	S	S
13 BENS	R	S	S	S	S	S
14 BENS	R	R	S	S	S	R
From dogs						
03 ELCH	R	S	S	S	S	R
03 BENS	S	S	S	S	S	S
06 BENS	S	S	S	S	S	S
10 BENS	S	S	S	S	S	S
03 ZAK	S	S	S	S	S	S
04 ZAK	S	S	S	S	S	S
05 ZAK	S	S	S	S	S	S
11 NAB	S	S	S	S	S	S
From owners						
P-02 ELch	S	S	S	S	S	S
P-03 ELch	S	S	S	S	S	S
P-06/07 ELch	S	S	S	S	S	S
P-06 BENS	S	S	R	S	S	S
P-09 BENS	S	S	S	S	S	S
P-10 BENS	S	S	S	S	S	S
P-11 BENS	S	S	S	S	S	S
P-15 BENS	S	S	S	S	R	S
P-17 BENS	S	S	R	S	S	S
P-02 BENF	S	S	S	S	S	S
P-01 BRA	S	S	S	S	S	S

Isolates in bold are methicillin resistant *S. aureus* (MRSA). FOX: ceftiofur; K: kanamycin; E: erythromycin; CIP: ciprofloxacin; DA: clindamycin; FA: fusidic acid; R: resistant; S: susceptible.

significant. The risk of nasal carriage of *S. aureus* was higher in dogs over 2 years of age compared to those ≤ 2 years of age (odds ratio= 9.49, 95% CI: 1.39- 69.36, $p= 0.03$).

Discussion

In this cross-sectional study, the rate of nasal

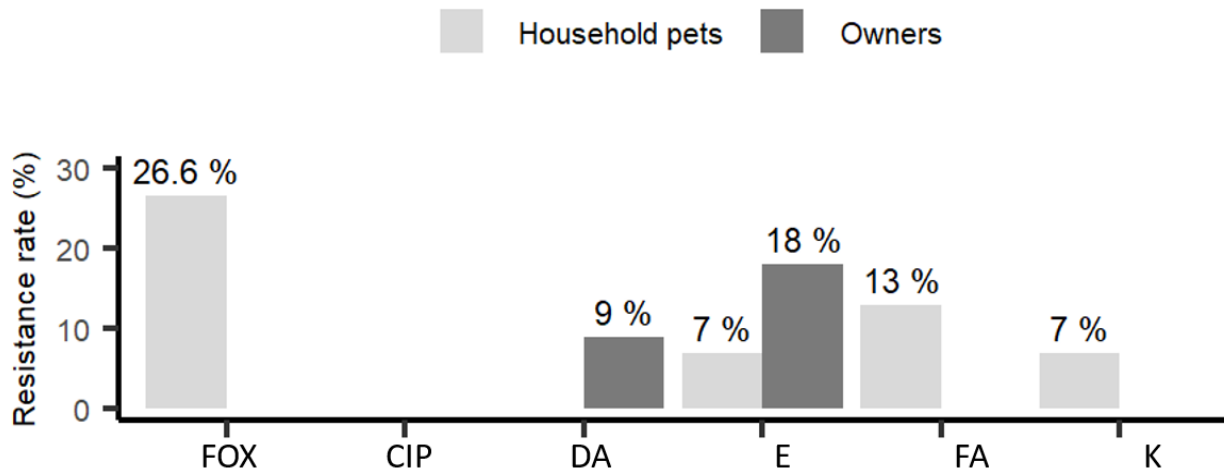


Figure 1) Antibiotic resistance rates of *Staphylococcus aureus* isolates isolated from household pets and their owners in Chlef, Algeria. FOX: Cefoxitin, CIP: Ciprofloxacin, DA: Clindamycin, E: Erythromycin, FA: Fusidic Acid, K: Kanamycin.

carriage of *S. aureus* and MRSA in household pets and their owners was estimated, the antibiotic resistance profiles of the isolates were determined, and the influence of several risk factors was assessed. This study results revealed a relatively high nasal carriage rate of *S. aureus* in dogs (27.6%), cats (22.6%), and their owners (22%).

Unlike several studies on clinical isolates of *S. aureus*, there is only one previous study by Mairi et al. (2019) on nasal carriage of *S. aureus* in pets and their owners in Algeria, which included samples from several provinces (excluding Chlef) and reported nasal carriage rates of 18.88, 15.43, and 13.25% in cats, dogs and owners, respectively [9]. Compared to the previous study in Algeria [9], the *S. aureus* carriage rate in the current study was similar in cats (22.6%) but higher in both dogs (27.6%) and household pet owners (22%).

The nasal carriage rate of *S. aureus* in cats in this study (22.6%) was close to that reported in Spain (25%) [8], but much higher than those recorded in Greece (0%), Saudi Arabia (2.4%), and Tunisia (5.9%) [8].

The *S. aureus* nasal carriage rate in dogs (27.6%) was comparable to that recorded in Bangladesh (25%) [17], but much higher than

those reported elsewhere (from 0 to 11%) [8], and lower than those reported in Nigeria (36.9%) [18] and Indonesia (48%) [19].

The nasal carriage rate of *S. aureus* among household pet owners (22%) was similar to those reported in the general population of Hong Kong and northern Germany [20, 21].

Regarding MRSA, the overall nasal carriage rate of MRSA in pets (6.6%) was in close agreement with that (4.3%) reported by Mairi et al. (2019) [9] in Algeria.

The MRSA carriage rate in dogs in this study (3.4%) was similar to those reported in Brazil (3.5%) [22] and Egypt (2.1%) [23], but higher than those reported in Australia (0%) [24], Taiwan (0.13%) [25], and Brazil (1.2%) [26], and lower than those registered in Indonesia (28%) [19] and Myanmar (47.7%) [27]. The nasal carriage rate of MRSA in cats in this study (9.6%) was comparable to that reported (6.63%) in Poland [28]; however, it was relatively higher than those (<2%) reported in many other studies [8, 29].

Comparison of *S. aureus* and/or MRSA nasal carriage rates between different studies should be interpreted with caution due to study-specific factors, such as the type of study population, health conditions, environmental factors, and *S. aureus*

Table 3) Risk factors for nasal carriage of *Staphylococcus aureus* in household pets and their owners in Chlef province, Algeria

		Risk Factor	Number of Samples	Number of Positives	P-Value
Dogs N=29	Sex	M	16	4 (25%)	.99
		F	13	4 (30.76%)	
	Age (years)	≤ 2	23	4 (17.39%)	.03*
		> 2	6	4 (66.66%)	
	Contact with other animals	Yes	18	7 (38.88%)	.10
		No	11	1 (9.09%)	
	Antibiotic administration	Yes	6	1 (16.66%)	.64
		No	23	7 (30.43%)	
	Vaccination	Yes	19	6 (31.57%)	.67
		No	10	2 (20%)	
Presence of infection	Yes	14	4 (28.57%)	.99	
	No	15	4 (26.66%)		
Cats N=31	Sex	M	20	5 (25%)	.99
		F	11	2 (18.18%)	
	Age (years)	≤ 2	26	5 (19.23%)	.56
		> 2	5	2 (40%)	
	Contact with other animals	Yes	18	2 (11.11%)	.09
		No	13	5 (38.46%)	
	Antibiotic administration	Yes	8	0 (0%)	.14
		No	23	7 (30.43%)	
	Vaccination	Yes	10	3 (30%)	.65
		No	21	4 (19.04%)	
Presence of infection	Yes	18	4 (22.22%)	.99	
	No	13	3 (23.07%)		
Household pets' owners N=50	Sex	M	32	6 (18.75%)	.49
		F	18	5 (27.77%)	
	Age (years)	≤ 25	23	3 (13.04%)	.28
		>25	27	8 (29.62%)	
	Owner of	Dog	24	6 (25%)	.88
		Cat	26	5 (19.23%)	
	Contact with other animals	Yes	25	7 (28%)	.49
		No	25	4 (16%)	
	Antibiotic administration	Yes	7	1 (14.28%)	.99
		No	43	10 (23.25%)	
Chronic disease	Yes	9	2 (22.22%)	.99	
	No	41	9 (21.95%)		
Recent hospitalisation	Yes	1	1 (100%)	.22	
	No	49	10 (20.40%)		

N: Number, M: Male, F: Female.

*: Odds Ratio (Age > 2 vs. Age ≤ 2 years) = 9.49 and 95% CI: 1.39 – 69.36.

identification methods.

One of the four MRSA isolates, obtained from a cat, was shown to possess PVL toxin genes (25%, 1 of 4), which is in line with a study in Saudi Arabia, reporting a prevalence of 26.7% for PVL-positive MRSA in cats [30]. Contrary to this study finding, several studies have reported higher carriage rates of PVL-positive MRSA in dogs compared to cats [24, 31, 32].

Nonetheless, this result is noteworthy because MRSA strains producing PVL toxin have been identified in many studies as CA-MRSA, causing severe soft tissue and skin infections in humans [33, 34].

It is currently unclear whether the PVL-positive MRSA isolate identified in this study belongs to a CA-MRSA clone; however, it is plausible that it is the case, since cross-transmission of MRSA between humans and animals is well documented [4, 5, 31].

While all MSSA isolates in this study displayed a similar antibiotic resistance profile, being susceptible to all antibiotics tested, the four MRSA isolates exhibited four different profiles, and one of which was MDR, indicating that they are different strains and supporting previous findings that MRSA strains have a strong potential to acquire resistance genes [35].

Among all the risk factors investigated in this study, only the age of dogs showed a significant association with *S. aureus* nasal carriage.

The absence of any significant association between the risk factors and the nasal carriage could be attributed to the lack of statistical power, which may be due to the small size of the pet and human populations studied.

Conclusion

Despite the limitations of this study, particularly the small sample size, the restricted geographical location, and the

lack of genotyping data, it provides evidence that household pets (dogs and cats) are a substantial reservoir of MSSA and MRSA in Algeria and therefore a potential risk to human health.

These findings stress the importance of active surveillance of MSSA and MRSA in healthy cats and dogs and highlight the need for raising awareness among pet owners and veterinarians about the risks they are exposed to during contact with animals colonized by MSSA or MRSA.

Acknowledgements

We would like to thank the participants in this study for allowing us to collect nasal swab samples and the veterinarians for their kindly assistance in collecting samples and associated data.

Ethical permissions: This study was approved by the Ethics Committee of the Algerian Thematic Research Agency for Health and Life Sciences, under the registration number ATRSSV387. Written informed consents were obtained from all participants.

Authors' contributions: Abla Djebbar, Hadjer Kaufa, Bouchra Goutal, Rachida Namoune, and Kahina performed the experimental parts of the study and analysed the results. Mohammed El Amine Bekara performed the statistical analysis. Abla Djebbar and Mohammed Sebahia conceived the study, supervised the research, analysed and interpreted the data, and wrote the manuscript.

Conflict of interests: None declared by authors.

Fundings: This research was funded by the Algerian Ministry of Higher Education and Scientific Research (DGRSDT/MESRS).

Consent to participate: Not applicable.

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