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COMMENSAL STAPHYLOCOCCUS SPP. FROM DOGS AND CATS AS RESERVOIRS OF ANTIMICROBIAL RESISTANCE GENES IN NORTHEASTERN BRAZIL: A PRELIMINARY SURVEY

STAPHYLOCOCCUS SPP. COMENSAIS DE CÃES E GATOS COMO RESERVATÓRIOS DE GENES DE RESISTÊNCIA ANTIMICROBIANA NO NORDESTE DO BRASIL: UM ESTUDO PRELIMINAR

STAPHYLOCOCCUS SPP. COMENSALES DE PERROS Y GATOS COMO RESERVORIOS DE GENES DE RESISTENCIA ANTIMICROBIANA EN EL NORESTE DE BRASIL: UN ESTUDIO PRELIMINAR

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ABSTRACT

Antimicrobial resistance (AMR) in commensal microorganisms from companion animals represents a growing challenge within the One Health framework, as the close coexistence between pets and humans facilitates the circulation of resistance genes across species. This study aimed to characterize *Staphylococcus* spp. isolated from the oropharynx of dogs and cats attended at veterinary clinics in the Metropolitan Region of Recife, Pernambuco, Brazil, focusing on both phenotypic and genotypic antimicrobial resistance profiles. Oropharyngeal samples from 20 animals (13 dogs and 7 cats) were cultured on Mannitol Salt Agar, and isolates were identified by MALDI-TOF mass spectrometry. Antimicrobial susceptibility was assessed using the disk diffusion method, and resistance genes were screened by PCR. Eleven *Staphylococcus* isolates were identified, including *S. aureus* (n=2), *S. felis* (n=2), *S. sciuri* (n=2), *S. warneri* (n=2), *S. haemolyticus* (n=1), *S. nepalensis* (n=1), and *S. simulans* (n=1). Erythromycin resistance predominated (6/11; 54.5%), and all resistant isolates exhibited inducible clindamycin resistance (D-test positive). The *blaZ*, *norA*, *norC*, and *tet(38)*

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genes were detected, while *mecA* and *mecC* were absent. These findings demonstrate the genetic diversity of *Staphylococcus* spp. colonizing the oropharynx of dogs and cats and reveal the silent circulation of antimicrobial resistance determinants in companion animals. The results reinforce the need for integrated AMR surveillance connecting human, animal, and environmental health sectors to prevent the dissemination of resistance within the One Health continuum.

Keywords: *Staphylococcus spp*. Companion Animals. Resistance Genes. One Health.

RESUMO

A resistência antimicrobiana (RAM) em micro-organismos comensais de animais de companhia representa um desafio crescente no contexto de Saúde Única (*One Health*), uma vez que a convivência próxima entre pets e humanos favorece a circulação e a persistência de genes de resistência entre espécies. Este estudo teve como objetivo caracterizar isolados de *Staphylococcus* spp. provenientes da orofaringe de cães e gatos atendidos em clínicas veterinárias da Região Metropolitana do Recife, Pernambuco, com foco nos perfis fenotípico e genotípico de resistência antimicrobiana. Amostras orofaríngeas de 20 animais (13 cães e 7 gatos) foram cultivadas em Ágar Sal Manitol, e os isolados foram identificados por espectrometria de massa MALDI-TOF. A susceptibilidade antimicrobiana foi avaliada pelo método de difusão em disco, e os genes de resistência foram pesquisados por PCR. Foram identificados onze isolados de *Staphylococcus*, incluindo *S. aureus* (n=2), *S. felis* (n=2), *S. sciuri* (n=2), *S. warneri* (n=2), *S. haemolyticus* (n=1), *S. nepalensis* (n=1) e *S. simulans* (n=1). A resistência à eritromicina foi predominante (6/11; 54,5%), e todos os isolados resistentes apresentaram resistência induzível à clindamicina (teste D positivo). Foram detectados os genes *blaZ*, *norA*, *norC* e *tet(38)*, enquanto *mecA* e *mecC* estavam ausentes. Os resultados demonstram a diversidade genética de *Staphylococcus* spp. colonizando a orofaringe de cães e gatos e revelam a circulação silenciosa de determinantes de resistência antimicrobiana em animais de companhia. Esses achados reforçam a necessidade de vigilância integrada de RAM, conectando os setores de saúde humana, animal e ambiental para prevenir a disseminação da resistência no âmbito da Saúde Única.

Palavras-chave: *Staphylococcus spp*. Animais de Companhia. Genes de Resistência. Saúde Única.

RESUMEN

La resistencia antimicrobiana (RAM) en microorganismos comensales de animales de compañía representa un desafío creciente en el contexto de Salud Única (One Health), dado que la convivencia cercana entre mascotas y humanos favorece la circulación y persistencia de genes de resistencia entre especies. Este estudio tuvo como objetivo caracterizar aislados de *Staphylococcus* spp. provenientes de la orofaringe de perros y gatos atendidos en clínicas veterinarias de la Región Metropolitana de Recife, Pernambuco, con enfoque en los perfiles fenotípico y genotípico de resistencia antimicrobiana. Se cultivaron muestras orofaríngeas de 20 animales (13 perros y 7 gatos) en Ágar Sal Manitol, y los aislados fueron identificados mediante espectrometría de masa MALDI-TOF. La susceptibilidad antimicrobiana se evaluó por el método de difusión en disco, y los genes de resistencia se investigaron por PCR. Se identificaron once aislados de *Staphylococcus*, incluyendo *S. aureus* (n=2), *S. felis* (n=2), *S. sciuri* (n=2), *S. warneri* (n=2), *S. haemolyticus* (n=1), *S. nepalensis* (n=1) y *S. simulans* (n=1). La resistencia a la eritromicina fue predominante (6/11; 54,5%), y todos los aislados resistentes presentaron resistencia inducible a la clindamicina (test D positivo). Se detectaron los genes *blaZ*, *norA*, *norC* y *tet(38)*, mientras que *mecA* y *mecC* estuvieron ausentes.Los resultados demuestran la diversidad genética de *Staphylococcus* spp. colonizando la orofaringe de perros y gatos y revelan la circulación silenciosa de determinantes de resistencia antimicrobiana en animales de compañía. Estos

hallazgos refuerzan la necesidad de vigilancia integrada de RAM, conectando los sectores de salud humana, animal y ambiental para prevenir la diseminación de la resistencia en el ámbito de la Salud Única.

Palabras clave: *Staphylococcus spp*. Animales de Compañía. Genes de Resistencia. Salud Única.

1 INTRODUCTION

The close relationship between humans and companion animals facilitates the transmission of microorganisms, including antimicrobial-resistant strains (Caddey *et al.*, 2025; O'Neill, 2014). This interspecies interaction promotes microbial exchange, hindering infection control and perpetuating resistance cycles (Guardabassi, 2016). Frequent antibiotic exposure in veterinary settings contributes to the dissemination of resistant *Staphylococcus* spp., posing a growing One Health challenge (Lax *et al.*, 2014).

Staphylococcus species inhabit skin, mucosa, and the respiratory tract of multiple hosts. In animals, these bacteria may cause severe infections, including wound infections and septicemia (Guo *et al.*, 2023). *S. pseudintermedius*, a coagulase-positive species frequently associated with canine and feline infections, represents a key example of AMR in companion animals (Abdullahi *et al.*, 2022). The spread of methicillin-resistant *S. aureus* (MRSA) and *S. pseudintermedius* further demonstrates zoonotic potential (Caddey *et al.*, 2025).

AMR dissemination arises through horizontal gene transfer mechanisms such as transformation, conjugation, and transduction (Bello-López *et al.*, 2019). Although resistance genes have been increasingly detected in *Staphylococcus* spp. from companion animals, important gaps remain regarding their maintenance, expression, and interspecies transmission. Addressing these gaps is essential to understand the role of pets as potential reservoirs within the One Health continuum. Therefore, this study aimed to characterize both the phenotypic and genotypic antimicrobial resistance profiles of *Staphylococcus* spp. isolated from the oropharynx of dogs and cats in Recife, Pernambuco, Brazil.

2 MATERIALS AND METHODS

2.1 SAMPLING AND ISOLATION OF *STAPHYLOCOCCUS* SPP.

Oropharyngeal swabs were obtained from 20 animals (13 dogs, 7 cats) through non-probabilistic convenience sampling (Sampaio, 1995). Five dogs were hospitalized, while the remaining animals were examined during routine visits. Samples were refrigerated during transportation to the Infectious Diseases Laboratory, Federal Rural University of Pernambuco. Swabs were plated on Mannitol Salt Agar and incubated at 37 °C for 24–48 h. Colonies were characterized by morphology, Gram staining, and catalase tests.

2.2 BACTERIAL DNA EXTRACTION

All colonies compatible with *Staphylococcus* spp. were reactivated on Brain Heart Infusion (BHI) Agar for genomic DNA extraction. After incubation at 35–37 °C for 24 hours,

DNA was extracted using a thermal lysis method according to the protocol described by Fan, Kleven, and Jackwood (1995). The extracted DNA was quantified and assessed for purity using a spectrophotometer, with absorbance readings taken at 260 nm.

2.3 MASS SPECTROMETRY (MALDI-TOF)

For species confirmation of *Staphylococcus*, the samples were sent to the Federal University of São Paulo, where identification was performed using mass spectrometry via the MALDI-TOF technique (Matrix-Assisted Laser Desorption/Ionization – Time of Flight Mass Spectrometry), with the Bruker Biotyper 4.1 database for spectrum comparison.

2.4 IN VITRO ANTIMICROBIAL SUSCEPTIBILITY TESTING

Phenotypic susceptibility was determined by disk diffusion (CLSI, 2024). Antibiotics tested included cefoxitin (30 µg), oxacillin (1 µg), erythromycin (15 µg), clindamycin (2 µg), gentamicin (10 µg), norfloxacin (10 µg), and tetracycline (30 µg). Isolates resistant to ≥3 antimicrobial classes were defined as multidrug-resistant (MDR). Inducible clindamycin resistance (ICR) was assessed using the D-test. Interpretive criteria followed veterinary standards (CLSI M100-S29).

2.5 DETECTION OF RESISTANCE GENES

The target genes, corresponding primers, and expected amplicon sizes used in this study are summarized in Table 1. Their inclusion allowed both the phenotypic and genotypic aspects of antimicrobial resistance to be assessed within the studied isolates. ATCC strains were used as positive control in the reactions and DNA-Free Water as a negative control.

Table 1

Primer sequences, amplicon sizes, and references used for PCR detection of antimicrobial resistance genes in Staphylococcus spp. isolates

Gene	**Primer sequence (5'→3')**	Amplicon size (pb)	Reference
blaZ	F:AAGAGATTTGCCTATGCTTC R:GGCAATATGATCAAGATAC	517	Sawant *et al.*, 2009
mecA	F: TGGTATGTGGAAGTTAGATTGGGAT	155	Nakagawa *et al.*,

	R: CTAATCTCATATGTGTTCCTGTATTGGC		2005
mecC	F: CATTAAAATCAGAGCGAGGC R: TGGCTGAACCCATTTTTGAT	188	Paterson *et al.*, 2012
norA	F: TGCAATTTCATATGATCAATCCC R: AGATTGCAATTCATGCTAAATATT	150	Truong-Bolduc, Zhang & Hooper, 2003
norC	F:AAATGGTTCTTCTAAGCGACCAA R: ATAAATACCTGAAGCAACGCCACC	200	Truong-Bolduc *et al.*, 2006
tet38	F: TTCAGTTTGGTTATAGACAA R: CGTAGAAATAAATCCACCTG	200	Truong-Bolduc *et al.*, 2006

3 RESULTS AND DISCUSSION

Of the 20 animals evaluated (13 dogs and 7 cats), 11/20 (55.0%) yielded isolates with characteristics consistent with *Staphylococcus* spp. Seven distinct species were identified: *S. aureus* (2/11; 18.2%), *S. felis* (2/11; 18.2%), *S. sciuri* (2/11; 18.2%), *S. warneri* (2/11; 18.2%), *S. haemolyticus* (1/11; 9.1%), *S. nepalensis* (1/11; 9.1%), and *S. simulans* (1/11; 9.1%). Coagulase-negative staphylococci predominated among the isolates, aligning with previous studies reporting their high prevalence in companion animals. The distribution of isolates according to host species is presented in Table 2.

Table 2

Identification of Staphylococcus spp. isolates recovered from oropharyngeal samples of dogs and cats

Isolate ID	Host	Species
1	Dog	*Staphylococcus aureus*
2	Cat	*Staphylococcus aureus*
3	Cat	*Staphylococcus felis*
4	Cat	*Staphylococcus felis*
5	Dog	*Staphylococcus sciuri*

6	Dog	*Staphylococcus sciuri*
7	Dog	*Staphylococcus warneri*
8	Dog	*Staphylococcus warneri*
9	Dog	*Staphylococcus haemolyticus*
10	Cat	*Staphylococcus nepalensis*
11	Cat	*Staphylococcus nepalensis*

The present study provides a preliminary yet relevant overview of *Staphylococcus* species colonizing the oropharynx of dogs and cats in northeastern Brazil, emphasizing their role as potential reservoirs of antimicrobial resistance (AMR). Despite the limited sample size, the findings highlight the diversity of commensal staphylococci in companion animals and reveal the presence of resistance determinants even among non-multidrug-resistant isolates.

The predominance of coagulase-negative staphylococci (CoNS), including *S. felis*, *S. sciuri*, and *S. warneri*, corroborates previous reports describing these species as part of the normal microbiota of pets and their increasing relevance as opportunistic pathogens (Nocera *et al.*, 2021; Abdullahi *et al.*, 2022; Phumthanakorn *et al.*, 2022). Although *S. pseudintermedius*, a major pathogen associated with canine infections and a known contributor to AMR dissemination, was not detected in this study, its absence may reflect the specific anatomical site sampled (oropharynx) and the small cohort evaluated. This limitation reinforces the need for broader surveillance, including nasal and dermal sites, to capture the full diversity of *Staphylococcus* spp. in companion animals (Caddey *et al.*, 2025).

The detection of *S. aureus* in both dogs and cats remains epidemiologically significant given its well-established zoonotic potential. Studies conducted in Brazilian veterinary hospitals have demonstrated overlapping strains of antimicrobial-resistant *S. aureus* among animals, owners, and veterinary staff, supporting the concept of bidirectional transmission within the One Health context (Leite *et al.*, 2023; Burke and Santoro, 2023). This interspecies exchange emphasizes the need for continuous molecular surveillance to identify emerging clones and resistance mechanisms.

Following species identification, the antimicrobial susceptibility profiles of the *Staphylococcus* isolates were determined according to CLSI (2024) guidelines. These results are summarized in Table 3, which integrates phenotypic resistance patterns with the corresponding genotypic findings. The combined analysis provides a comprehensive overview of resistance behavior in the isolates. Phenotypic analysis revealed erythromycin

resistance in 6/11 (54.5%) isolates, and all resistant isolates (6/6; 100%) exhibited inducible clindamycin resistance (ICR) confirmed by the D-test. Dual resistance to additional antimicrobials was observed in 2/11 (18.2%) isolates, one resistant to gentamicin and one to tetracycline, while 1/11 (9.1%) showed concurrent resistance to oxacillin and tetracycline. Another isolate (1/11; 9.1%) was resistant only to tetracycline.

Erythromycin resistance was the most prevalent phenotype observed (6/11; 54.5%), and all erythromycin-resistant isolates exhibited inducible clindamycin resistance (ICR). This finding aligns with previous investigations showing that the D-test remains a critical diagnostic tool for detecting inducible *erm*-mediated resistance (Delialioglu *et al.*, 2005). Although *erm* genes were not directly targeted in this study, the universal detection of the ICR phenotype suggests their likely presence and warrants their inclusion in future genotypic assays to strengthen phenotypic–genotypic correlations.

Genotypic characterization detected the presence of *blaZ*, *norA*, *norC*, and *tet(38)* genes. Specifically, one *S. aureus* isolate carried *blaZ*, *norA*, and *norC* simultaneously (1/11; 9.1%), and one *S. warneri* isolate harbored *tet(38)* (1/11; 9.1%), despite exhibiting no phenotypic tetracycline resistance. The *mecA* and *mecC* genes were not detected (0/11; 0%), indicating the absence of methicillin-resistant staphylococci among the analyzed isolates.

Overall, the comparison between phenotypic and genotypic data, as shown on Table 3, showed moderate agreement, as some isolates harbored efflux-associated genes (*norA*, *norC*, *tet(38)*) without corresponding phenotypic resistance. This highlights the potential for silent carriage of antimicrobial resistance determinants among commensal *Staphylococcus* species in companion animals.

Table 3

Phenotypic and genotypic characterization of antimicrobial resistance in Staphylococcus spp. isolates from veterinary clinical samples

Isolates	Species	Phenotypic resistance	Genotypic resistance
1	*Staphylococcus aureus*	ERY	-
2		ERY	*norA*, *norC* e *blaZ*
3	*Staphylococcus felis*	-	-
4		-	-

5	*Staphylococcus sciuri*	ERY and GEN	-
6		ERY and TET	-
7	*Staphylococcus warneri*	TET	-
8		-	*tet38*
9	*Staphylococcus haemolyticus*	ERY	-
10	*Staphylococcus nepalensis*	OXA and TET	-
11	*Staphylococcus simulans*	-	-

Legend: ERY – erythromycin; GEN – gentamicin; TET – tetracycline; OXA – oxacillin.

The absence of *mecA* and *mecC* in the isolates evaluated is consistent with reports of low prevalence of methicillin-resistant staphylococci among commensal animal populations in Brazil (Alcântara *et al.*, 2023; Souza *et al.*, 2024). Nevertheless, the identification of *blaZ* (β-lactamase production) and efflux-associated genes *norA*, *norC*, and *tet(38)*, even in isolates lacking phenotypic resistance, underscores the silent circulation of AMR determinants in the microbiota. This dissociation between genotype and phenotype has been attributed to regulatory mechanisms that modulate efflux pump expression under specific environmental or antibiotic stress conditions (Truong-Bolduc *et al.*, 2006; Marco-Fuertes *et al.*, 2024).

The limited number of isolates represents a constraint acknowledged. However, the molecular detection of resistance genes in non-pathogenic *Staphylococcus* spp. obtained from routine clinical environments suggests that these microorganisms may serve as genetic reservoirs, contributing to horizontal gene transfer and the broader environmental resistome (Bello-López *et al.*, 2019). Expanding the sampling to include different body sites, temporal replicates, and molecular typing methods such as multilocus sequence typing (MLST) or whole-genome sequencing (WGS) would greatly enhance the epidemiological resolution of future studies.

4 CONCLUSIONS

From a One Health perspective, these findings emphasize that companion animals,

through their close and constant interaction with humans, can act as silent reservoirs and disseminators of antimicrobial resistance genes. Even in the absence of multidrug-resistant phenotypes, the presence of resistance determinants in commensal *Staphylococcus* species highlights a hidden risk for cross-species transmission. Strengthening molecular surveillance networks and antimicrobial stewardship in veterinary medicine is essential to mitigate the spread of resistance across environmental and species boundaries, ensuring a unified response to the global AMR challenge.

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