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VETERINARY MICROBIOLOGY - RESEARCH PAPER

Occurrence of antimicrobial-resistant *Staphylococcus aureus* in a Brazilian veterinary hospital environment

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Abstract

Antimicrobial resistance is a threat to public health. The emergence of antibiotic-resistant *Staphylococcus aureus* represents a priority for the implementation of preventive measures. The objective was to isolate *S. aureus* in humans, animals, and animal health care environment, and to characterize the genotypic and phenotypic profile of antimicrobial resistance in these isolates. We isolated *S. aureus* from staff, animals, and environment of a veterinary hospital, and identified their antimicrobial resistance profiles. Samples were collected from 20 humans, 13 animals, 14 surfaces, 8 mobile phones, and 7 veterinarians' stethoscopes by using sterile swabs. *S. aureus* was isolated by culturing on mannitol salt agar and preliminary identification was done by Gram staining and catalase test. Subsequently, a polymerase chain reaction was performed for species confirmation and investigating their antimicrobial-resistant genotypic profiles. Phenotypic profiles of resistant isolates were determined using the disk-diffusion technique. Ten *S. aureus* isolates were recovered from 5/20 humans (25%), it was also recovered from 2/13 animals (15.38%), including 1 dog and 1 cat, and from 1/14 of surfaces (7.14%). The oxacillin-susceptible *mecA*-positive *Staphylococcus aureus* phenotype was identified in a feline. Most of the isolates carried at least two resistance genes of different antimicrobial classes, with 90% (9/10) presenting the gene *blaZ*, with 10% (1/10) presenting the gene *mecA*, 20% (2/10) presenting *tet38*, 10% (1/10) presenting *tetM*, 90% (9/10) presenting *norA*, 50% (5/10) presenting *norC*, 10% (1/10) presenting *ermA*, and 60% (6/10) presenting *ermB*. In antibiograms, resistance to penicillin was identified in all the isolates, resistance to erythromycin was identified in 80% (8/10), and all the isolate's resistance to erythromycin presented erythromycin-induced resistance to clindamycin. Antimicrobial resistance in the veterinary hospital requires attention due to the risk of interspecies transmission, gene transfer between bacteria that colonize companion animals and humans and, can make antimicrobial therapy difficult.

Keywords Canines · Disc-diffusion · Humans · Felines · OS MRSA · Genotypic profile

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Introduction

Antimicrobial resistance is a major threat to public health and global development [1]. Methicillin-resistant *Staphylococcus aureus* (MRSA) has been reported as a pathogen of global importance and priority for the implementation of preventive measures [2]. Human healthcare suffers from the impact of MRSA infection worldwide. In Latin America, MRSA infections resulted in more than a six-fold increase in the cost of antimicrobial therapy and a three-fold increase in the hospitalization frequency, accounting for > 45% mortality rate [3]. In veterinary medicine, an increase in the number of MRSA isolates has been reported in dogs and cats [4, 5] Besides, antimicrobial resistance in *S. aureus* is not restricted

to beta-lactams. The resistance patterns are outlined by the emergence of distinct strains that acquired resistance mechanisms to different classes of antimicrobials [6].

The efficiency of resistance genes acquisition through the transfer of mobile genetic elements in *S. aureus* accelerates the dissemination of clones resistant to most diverse antimicrobials. Genomic assays propose that genetic determinants of resistance are shared between staphylococcal species that colonize different environments and hosts [6, 7]. *S. aureus*, as well as other species of the genus and other genera, act as gene reservoirs, posing a threat to human and animal health. Close contact between humans and their animal companion reinforces the risk of mutual transfer of bacterial strains, especially resistant ones, as well as genetic exchanges involving said bacteria that colonize humans and their pets [5, 8].

In the world, few studies have aimed to identify the colonization of *S. aureus* in dogs and cats and associated antimicrobial resistance profiles; and, in Brazil, investigations are limited to the South and Southeast regions [9, 10]. Thus, the research aims to identify the presence of antimicrobial-resistant *S. aureus* in a veterinary hospital environment.

Material and methods

Sampling

Samples were collected from October 2021–to December 2021 at the Veterinary Hospital of the Department of Veterinary Medicine (HOVET-DMV) in the UFRPE, situated in the city of Recife, Pernambuco, Brazil.

Sterile swabs were used to collect samples from veterinarians and their instruments, dogs, cats, and their respective owners in the ambulatory care environment. In this research, samples were collected from 20 humans: 8 veterinarians and 12 tutors. Swabs were collected from hands (HS) and from nostril (NS), resulting in 20 HS and 20 NS. For the animals, samples were obtained from oropharyngeal swabs (OPS) of 10 dogs and 3 cats. Samples were also collected from 14 surfaces (SS), 13 of which were ambulatory tables and 1 weighing scale. Finally, samples were collected from objects owned by veterinarians, 8 cell phones (CS) and 7 stethoscopes (STS). The collections took place after the clinical consultations before any type of sanitization of the hands of the professionals and tutors, as well as of the fomites was performed.

Isolation and preliminary identification of *S. aureus*

Bacterial isolation was performed by plating swabs on mannitol salt agar (Difco Laboratories Inc., Detroit, USA). The plates were incubated in a bacteriological incubator at 37 °C (± 1 °C) for 24–48 h. Subsequently, bacterial growth was verified, and colonies were selected by Gram staining and catalase test [11]. The selected colonies were subcultured on mannitol salt agar to obtain confluent growth. A portion of bacterial growth was used for DNA extraction and the remaining culture was suspended in brain heart infusion broth (BHI; Difco Laboratories Inc., Detroit, USA) tubes, maintained at 37 °C for 24 h, and finally preserved at – 80 °C in the presence of 20% glycerol.

DNA extraction and *S. aureus* confirmation

Thermal extraction of the DNA was performed according to the methodology described by Fan, Kleven, and Jackwood [12]. The DNA obtained was quantified and analyzed for purity using a spectrophotometer (Thermo Fisher Scientific, MA, USA), with absorbance at 260 nm [13]. Molecular identification of *S. aureus* species was done by polymerase chain reaction (PCR) using the species-specific gene *nuc* (Table 1) [14]. Strain ATCC 43300 *S. aureus* subsp. *aureus* was used as a positive control and DNA-free water (QIAGEN, Hilden, Germany) as a negative control.

Table 1 Analyzed resistance genes, primer sequences, amplicon sizes in base pairs (bp), and respective references

Gene	Primer sequence (5′-3′)	Bp	References
Nuc	R: AGCCAAGCCTTGACGAACTAAGC F: GCGATTGATGGTGATACGGTT	279	[14]
blaZ	R: GGCAATATGATCAAGATAC F: AAGAGATTTGCCTATGCTTC	517	[15]
mecA	R: CTAATCTCATATGTGTTCCTGTAT TGGC F: TGGTATGTGGAAGTTAGATTGGGA T	155	[16]
mecC	R: TGGCTGAACCCATTTTTGAT F: CATTAAAATCAGAGCGAGGC	188	[17]
tetM	R: CGGTAAAGTTCGTCACACAC F: GTGGACAAAAGGTACAACGAG	406	[18, 19]
tetL	R: GTATCCCACCAATGTAGCCG F: TCGTTAGCGTGCTGTCATTC	267	[19, 20]
norA	R: AGATTGCAATTCATGCTAAATATT F: TGCAATTTCATATGATCAATCCC	150	[21]
norC	R: ATAAAATACCCTGAAGCAACGCCA CC F: AAATGGTTCTTCTAAGGCACCAA	200	[22]
tet38	R: CGTAGAAATAAATCCACCTG F: TTCAGTTTGGTTATAGACAA	200	[23]
ermA	R:GCCTGTCGGAATTGG F: GCGGTAAACCCCTCTGAG	434	[24]
ermB	R:GGAACATCTGTGGTATGGCG F:CATTTAACGACGAAACTGGC	425	[25]

Genotypic profiling of antimicrobial resistance

To detect beta-lactam resistance genes in *S. aureus* isolates, PCR was performed using primers for the genes *blaZ*, *mecA*, and *mecC*. PCR was also performed to detect the tetracycline resistance genes *tetM*, *tetL*, *tet 38*, quinolone resistance genes *norA and norC*, and the macrolide resistance genes *ermA* and *ermB* (Table 1) ATCC® strains were used as positive controls and DNA-free water was used as a negative control. Thermocycler standards are according to the authors of Table 1.

Antimicrobial susceptibility test

The disk-diffusion assay was performed to evaluate the antimicrobial resistance profile of *S. aureus* isolates. The susceptibility testing was performed on Mueller–Hinton agar plates using the inoculum in suspension equivalent to 0.5% of the McFarland scale, according to the Standards Institute M100 Clinical and Laboratory Standards Institute [26]. *S. aureus* strain ATCC →25,923 was used as positive quality control in all the experiments.

The plates were incubated at 37 °C (± 1 °C) for 16–18 h. All the readings were recorded according to the M100 document. Following CLSI guidelines, we used the cefoxitin disc test (CFO, 30 µg) to detect oxacillin resistance in *S. aureus* [26].

To detect resistance to other beta-lactams, ceftiofur (CFT, 30 µg), penicillin (PEN, 10 µg), and penicillin + novobiocin (PNM, 40 µg) discs were used. In addition, to detect resistance toward other antimicrobials, discs of ciprofloxacin (CIP, 05 µg), clindamycin (CLI, 2 µg), chloramphenicol (CLO, 30 µg), doxycycline (DOX, 30 µg), erythromycin (ERI, 15 µg), gentamicin (GEN, 10 µg), linezolid (LNZ, 30 µg), neomycin (NEO, 30 µg), rifampicin (RIF, 30 µg), Sulfazotrim (sulfamethoxazole + trimethoprim, SUT, 25 µg), and tetracycline (TET, 30 µg) were used. Enrofloxacin discs (ENO, 05 µg) were also used in animal samples.

Results

Of the 110 bacterial isolates, 10 were identified as *S. aureus* by gene-specific (*nuc*) PCR (Table 2). The species was isolated from the nasal cavities of 5/20 (25%) humans, while no isolates were identified in hand swabs collected from the same individuals. Considering the animals, 2/13 (15.38%) were colonized by *S. aureus*, one feline, and one canine. The microorganism was also isolated from 1/14 (7.14%) surfaces and was not isolated from stethoscopes and mobile phones.

Isolates C and D, obtained from the same veterinarian, were considered separate samples whereas they showed different genotypic profiles of antimicrobial resistance (Table 4). Isolates H and I, obtained from the same animal owner, were considered separate samples, whereas they showed different phenotypic and genotypic profiles of antimicrobial resistance (Table 4).

Table 2 *S. aureus* isolates recovered from humans, animals, and the environment

Sample	Swab type	Origin
A	Oropharyngeal swabs	Feline (sample of animal 1)
B	Nasal swabs	Veterinarian (professional sample 3)
C	Nasal swabs	Veterinarian (professional sample 6)
D	Nasal swabs	Veterinarian (professional sample 6)
E	Surface swabs	Ambulatory table (surface sample 6)
F	Nasal swabs	Tutor (sample of tutor 9)
G	Oropharyngeal swabs	Canine (sample from animal 9)
H	Nasal swabs	Tutor (sample of tutor 10)
I	Nasal swabs	Tutor (sample of tutor 10)
J	Nasal swabs	Tutor (sample of tutor 12)

The positive ambulatory table (surface 6) for *S. aureus* was used by a veterinarian who also tested positive (professional number 6). In addition, a dog (animal number 9) and its owner (tutor number 9) were both positive for *S. aureus* (Table 2).

The analysis of beta-lactam-resistance genes (Table 3) revealed the presence of the gene (*blaZ)* in 9/10 (90%) of *S. aureus* isolates. The *mecA* gene was only detected in one feline *S. aureus* isolate; however, phenotypic resistance was not observed, characterized as oxacillin-susceptible *mecA*-positive *Staphylococcus aureus* (OS-MRSA) phenotype.

Tetracycline resistance genes *tetM* and *tet38* were detected in 1/10 (10%) and 2/10 (20%) of the isolates, respectively. The gene *tetL* was not detected (Table 4). Molecular detection of the multidrug efflux system was also included in the search for quinolone resistance genes. Quinolone resistance genes *norA* and *norC* were found in 9/10 (90%) and 5/10 (50%) of the isolates, respectively. Macrolide resistance genes *ermA* and *ermB* were detected in 1/10 (10%) and 6/10 (60%) of the isolates, respectively. The genes responsible for resistance to quinolones and macrolide were detected isolated from the environment, humans, and animals (Table 4).

Resistance genes against beta-lactams, quinolones, and macrolide were detected in isolates recovered from animals and humans, as well as the veterinary environment. Tetracycline resistance genes were also detected in isolates from humans. In disk-diffusion tests, there was no evidence of resistance to quinolones (ciprofloxacin and enrofloxacin) and tetracyclines (doxycycline and tetracycline). In phenotypic assays, resistance to erythromycin and erythromycin-induced resistance to clindamycin was observed in 8/10 (80%) of isolates: one recovered from the environment, one isolated from a feline, and six obtained from humans.

Table 3 Genotypic and phenotypic profiles of beta-lactam-resistant *S. aureus* isolates

Sample	Origin	Beta-lactam resistance gene	Antibiogram			
			PEN	CFO	CTF	PNM
E	Ambulatory table	*blaZ*	R	S	S	S
A	Feline	*blaZ* and *mec(A)*	R	S	S	S
G	Canine	*blaZ*	R	S	S	S
B	Veterinarian	*blaZ*	R	S	S	S
C	Veterinarian	*blaZ*	R	S	S	S
D	Veterinarian	*blaZ*	R	S	S	S
F	Tutor	*blaZ*	R	S	S	S
H	Tutor	*blaZ*	R	S	S	S
I	Tutor	*blaZ*	R	S	S	S
J	Tutor	*blaZ*	R	S	S	S

CFO cefoxitin, *CFT* ceftiofur, *PEN* penicillin, *PNM* penicillin + novobiocin, *R* resistant, *S* sensitive

Table 4 Genotypic and phenotypic profile of *S. aureus* isolates resistant to other antimicrobials

Sample	Origin	Resistance genes for other antimicrobials	Antibiogram											
			ERI	CLI	CIP	ENO	RIF	TET	CLO	DOX	GEN	NEO	LNZ	SUT
E	Ambulatory table	*norA and ermB*	R	iR	S	S	S	S	S	S	S	S	S	S
A	Feline	*norA*	R	iR	S	S	S	S	S	S	S	S	S	S
G	Canine	*norA*, *norC and ermB*	S	S	S	S	S	S	S	S	S	S	S	S
B	Veterinarian	*norA*, *norC*, *tet38 and ermB*	R	iR	S	S	S	S	S	S	S	S	S	S
C	Veterinarian	*norA*	R	iR	S	S	S	S	S	S	S	S	S	S
D	Veterinarian	*norA*, *norC and ermB*	R	iR	S	S	S	S	S	S	S	S	S	S
F	Tutor	*norA, tetM, ermA and ermB*	R	iR	S	S	S	S	S	S	S	S	S	S
H	Tutor	*norA*, *norC* and *tet38*	S	S	S	S	S	S	S	S	S	S	S	S
I	Tutor	*norA, norC and ermB*	R	iR	S	S	S	S	S	S	S	S	S	S
J	Tutor	No gene	R	iR	S	S	S	S	S	S	S	S	S	S

CIP ciprofloxacin, *CLI* clindamycin, *CLO* chloramphenicol, *DOX* doxycycline, *ENO* enrofloxacin, *ERI* erythromycin, *GEN gentamicin*, *LNZ* linezolid, *NEO* neomycin, *RIF* rifampicin, *SUT* Sulfazotrim (sulfamethoxazole + trimethoprim), *TET* tetracycline, *R* resistant, *iR* induced resistant, *S* sensitive

Discussion

In studies carried out in Africa, North America, Europe, and Oceania, the occurrence of *S. aureus* ranged from 10.4 to 34% and 8.1 to 21% in dogs and cats, respectively [4, 5, 27–29], similar to the results obtained in the present study. However, the variations in results may be attributed to the different sampling and isolation methods employed in different studies [30]. Other factors include the health status of the sampled animals [31]; history of antimicrobial therapy, surgical procedures, and hospitalizations [32, 33]; rearing style (free-living or domiciled animals in close contact with their owners) [4, 34]; and coexistence with MRSA positive humans, or individuals who work in either public or animal healthcare field [4, 35].

In routine clinical and laboratory settings, it is unusual to identify S. *aureus* species. Most scientific studies report only the occurrence of the *Staphylococcus* genus, or even classification into coagulase-negative and coagulase-positive Staphylococci [36, 37]. Previous data regarding *S. aureus* colonization of companion animals have focused on dogs and have reported low colonization rates [5, 27, 28, 30]. In Brazil, some investigations even demonstrated the non-recovery of this bacterial species [36], and others reported detection in 1.97% and 15.8% [9, 10, 38], while data on felines are scarce, bacterial recovery occurred in 4.7% of these [39].

A low colonization rate of *S. aureus* was also expected in animals. Considering that dogs and cats are preferentially colonized by other species such as *S. epidermidis, S. felis*, *S. intermedius*, *S. pseudintermedius*, *S. schleiferi*, and *S. simulans*, *S. aureus* is not frequently isolated [34, 40]. Furthermore, the dynamics of colonization by *S. aureus* of these animals, especially dogs, occur intermittently [4, 28, 34, 36]. Colonization by *S. aureus* and

MRSA of humans has a variable rate between populations [41] owing to multiple factors, such as frequency of contact and time of exposure to host animals. Still, some studies evidenced a high percentage of colonization by *S. aureus* and MRSA in owners and people who work in close contact with animals, including veterinarians and other members involved in animal health care [42, 43].

MRSA isolates were not recovered from human samples. *S. aureus* was isolated from 25% (2/8) of veterinarians. In Brazil, no previous studies are reporting the colonization of pet veterinarians by *S. aureus*. Internationally, most studies report occurrence in veterinarians in contact with farm animals, demonstrating recovery of the bacteria in 64% to 75% of individual [44, 45]) In Italy [46], 25% of pet veterinarians were colonized by *S. aureus*, and MRSA was present in 1.6% of them. Australia and the UK had the highest MRSA prevalence in pet veterinarians, 16% and 17.9%, respectively [47].

Studies carried out in Brazil have focused on the sampling of farm milkers on rural properties [48, 49]. There is a lack of information regarding the owners of dogs and cats. However, frequency and time of contact with pets and sharing home environment, isolates are expected to be recovered from owners [42, 43]. In this investigation, recovery occurred in 25% (3/12) of these, similar to what was recently found in the country [9].

Pathogenic isolates were only recovered from nasal swabs in humans. The presence of bacteria in the nostrils was expected because these are the main anatomical site of colonization [27, 41]. No isolates were identified in samples from the hands of a veterinarian. This may be linked to the frequency of hand sanitizing and the use of gloves in occupational activities. Healthcare professionals can contribute to intra-and inter-species transmission, environmental dissemination through contaminated hands, or airborne transmission [50]. Accordingly, it is necessary to reinforce hand hygiene practices, which represent one of the main factors to reduce the incidence of healthcare-related infections and transmission of nosocomial pathogens [51] in human and veterinary medicine [52, 53]. The lack of *S. aureus* isolates in swabs from owner's hands probably occurred due to hygiene measures implemented during the current pandemic caused by SARS-CoV2, such as increased frequency of hand hygiene procedures and the use of alcohol-based gels [54].

Environmental surfaces and equipment used during animal handling, and even cell phone of tutors and veterinarians, are contaminated by a range of pathogens; therefore, they have an important epidemiological impact on the spread of microorganisms [55–58]. However, in this study, *S. aureus* was isolated from only one analyzed surface and was not isolated from stethoscope or cell phone samples, in agreement with other investigations that demonstrated the difficulty of bacterium survival on inanimate objects [59].

S. aureus was isolated from an outpatient veterinary table and from a veterinarian using the table. Furthermore, the bacterium was isolated from a dog and its owner. Bacterial transmission was not investigated in this study; however, transmission of *S. aureus* between animals and humans has already been reported in molecular epidemiological studies in other countries [60, 61] and in Brazil [9, 10].

To the best of our knowledge, this is the first report describing the OS-MRSA detection in company animals from the first report on the detection of OS-MRSA in animals in the Northeast region of Brazil. This phenotype has been reported in numerous countries [62–65] and is associated with human health care and community environments. It exhibits a high prevalence and presents a challenge for clinical management of staphylococcal infections [66]. It is assumed that in surveillance studies that focus on the presence and spread of MRSA among companion animals and their human contacts, OS-MRSA phenotype is neglected, its spread occurs silently, and isolates can be misinterpreted as methicillin-susceptible *S. aureus* (MSSA) [65].

In clinical microbiology laboratories, the resistance profiles of specimens are established by phenotypic testing. However, the search for resistance genes is the gold standard technique. Identification at the genetic level is limited due to its complex and costly techniques and the greater demand for financial resources. Therefore, routine activity in clinical laboratories is supported by methodologies involving minimum inhibitory concentration or disk-diffusion assays, which restricts the identification of OS-MRSA phenotype, misinterpreted as MSSA [62–65].

Incorrect identification of OS-MRSA phenotype leads to failure in the treatment of MRSA infections. OS-MRSA has the potential to develop resistance to beta-lactams due to possible expression of genes *mecA* or *mecC*. Antimicrobial therapies are based on susceptibility results, and not on laboratory identification of the OS-MRSA profile, which can lead to treatment failure and, potentially, the patient's death [64]. A previous study conducted in the same municipality as this research showed the isolation and dissemination of OS-MRSA profiles in people associated with human health care [67]. It is necessary to expand epidemiological assessments and studies concerning virulence and dissemination factors of OS-MRSA. Additionally, it is necessary to pay attention to this profile in companion animals, since this phenotype's transmission may occur silently between human and their pets (dogs and cats) [9].

Analysis of genotypic profiles revealed the presence of genes that were not expressed in the phenotypic evaluation. Certain environmental stress factors may lead to defects in the regulatory process of gene expression. Regarding such defects, certain genes may not be expressed, or, despite expression, the levels may be too low to ensure the growth and survival of the microorganism [68].

Resistance associated with efflux pumps only occurs when their structural genes are amplified or overexpressed because of regulatory mechanisms, such as occurs with the gene *norA* to synthesize NorA protein [69]. Furthermore, alterations at the transcriptional level can reduce the effectiveness of NorA and NorC proteins. These alterations are responsible for modifying polypeptide sequences in efflux pumps [70]. Moreover, antimicrobial resistance developed through this mechanism reveals increased expression at sites of infection, not necessarily being similar to in vitro activities [71]. *S. aureus* can exhibit different patterns of susceptibility according to the expression of efflux pump genes, even under pressure from the same antimicrobial agent [72].

Regarding tetracycline resistance, the involvement of two different mechanisms has been recognized. Ribosomal protection is encoded is encoded by *tetM*, *tetO*, *tetS*, and *tetW* genes, and the active efflux resulting from the expression of *tetK*, *tetL*, *tet38*, and *tet42* genes [73, 74]. The *tetM* and *tet38* genes have been detected, but neither were expressed. The occurrence of frameshift mutations results from the insertion or loss of bases and alterations to the machinery of expression of *tetM* [75] as well as the upregulated expression of *tet38* at sites of infection [76, 77]. Therefore, in vitro laboratory conditions limit the results, which differ from those found in vivo conditions [78].

Erythromycin resistance mediated by the erm genes results from ribosomal modification [79]. Most of the isolates in our study considered resistant to erythromycin in the antibiogram technique carried at least one erythromycin-resistance gene, in agreement with the results obtained by Duran et al. [80]. Although, the frequency of the *ermB* gene was higher in our study than those obtained by Lim et al. [81] and Martineau et al. [82], who detected the higher frequency of the *ermA* gene.

Resistance genes have epidemiological importance because of the risk of expression at a given moment. Moreover, resistance genes can be transmitted among co-colonizing bacteria through the mobile genetic elements that carry such genes [83].

This study is one of the few developed in the country, being the first in the Northeast region of Brazil that investigated the occurrence of antimicrobial-resistant *S. aureus* in animals, humans, and the environment at an animal care facility. New investigations focusing on molecular epidemiology must be carried out to understand the role of dogs and cats as potential reservoirs of *S. aureus* and its resistant specimens, as transmission may occur from animals to humans. Therefore, further studies to evaluate the risk factors involving interspecies transmission need to be conducted.

Conclusion

The occurrence of the OS-MRSA phenotype and *Staphylococcus aureus* isolates carrying resistance genes to different classes of antimicrobials recovered from dogs and cats, humans, and veterinary environment reinforce the need to implement prevention strategies in veterinary practices to combat antimicrobial resistance.

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Declarations

Ethics approval All experimental procedures were in accordance with the ethics principles accepted by the Ethics Committee for the Use of Animals of the Federal Rural University of Pernambuco (UFRPE), license number 1466270721, as well as by the Plataforma Brazil, license number 46827221.7.0000.9547.

Consent for publication All authors approved the version to be published.

Conflict of interest The authors declare no competing interests.

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