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NASAL COLONIZATION BY GOLDEN-PIGMENTED COAGULASE-POSITIVE *STAPHYLOCOCCUS* SPP. IN DOMESTIC CATS IN THE PROVINCE OF ESPÍRITO SANTO, BRAZIL: ANTIMICROBIAL RESISTANCE PROFILES AND ONE HEALTH IMPLICATIONS

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ABSTRACT

Staphylococci capable of producing golden-pigmented colonies, commonly associated with *Staphylococcus aureus* and other coagulase-positive species such as *Staphylococcus pseudintermedius*, are significant opportunistic pathogens in both veterinary and human medicine. This research aimed to determine the prevalence of such staphylococci in the nasal cavity of cats attended at a veterinary clinic in Bom Jesus do Norte, Province of Espírito Santo, Brazil, and to characterize their antimicrobial susceptibility profiles. Nasal swabs were collected from 45 clinically healthy cats that had not received antimicrobials in the preceding six months. Bacterial isolation, phenotypic identification, and antimicrobial susceptibility testing were performed according to EUCAST guidelines using the Kirby-Bauer disk diffusion method. Golden-pigmented, coagulase-positive *Staphylococcus* spp. were isolated from 15 cats (33.3% prevalence). Among these isolates, 66.6% (10/15) were resistant to cefoxitin, indicating methicillin resistance. Resistance to ampicillin and penicillin was observed in 93.3% of isolates, while macrolides, lincosamides, and chloramphenicol showed variable resistance. Notably, all isolates remained fully susceptible to ciprofloxacin, linezolid, tetracycline, and rifampicin. Network analysis of co-resistance patterns revealed a central cluster of β-lactam resistance tightly linked to other antimicrobial classes, consistent with a multidrug-resistant phenotype. These findings highlight the circulation of methicillin-resistant staphylococci in apparently healthy pet cats in the southern region of the Province of Espírito Santo, underscoring their potential role as reservoirs of antimicrobial resistance genes within the One Health framework. The high susceptibility to non-β-lactam antimicrobials supports their judicious use in empirical therapy, while the elevated resistance to methicillin prevalence calls for enhanced surveillance and antimicrobial stewardship in veterinary practice.

KEYWORDS: *Staphylococcus*; methicillin-resistant; cats; antimicrobial resistance; nasal colonization; One Health.

INTRODUCTION

The relationship between humans and domestic cats (*Felis catus*) is not only ancient: it is deeply intertwined with the trajectory of human civilization itself. Archaeological evidence indicates that this partnership began at least 11,000 years ago in the Fertile Crescent (modern-day Syria and Iraq), where cats were initially valued for their role in controlling rodent populations in early agricultural settlements.[1,2,3] Over time, this interaction evolved from a utilitarian alliance into a symbolic and affective bond, particularly in ancient Egypt, where cats were revered as sacred beings, associated with deities, and integrated into the core of social and religious life.[4,5] In contemporary societies, domestic cats are often regarded as family members, sharing not only living spaces but also furniture such as beds and sofas, daily routines, affectionate interactions, and even food. This prolonged and intimate cohabitation, however, extends beyond emotional ties: it creates a shared microbial ecosystem. Recent studies demonstrate that cats and their human caregivers exhibit more similar microbial profiles to each other than to individuals from other households, reflecting continuous exchange of commensal microorganisms as well as bacteria with pathogenic potential.[6,7]

A critical example of this dynamic is nasal colonization by bacteria of the genus *Staphylococcus*, particularly those classified as "golden staphylococci", a term historically associated with *Staphylococcus aureus* but also applicable to certain coagulase-negative species such as *Staphylococcus pseudintermedius* and *Staphylococcus schleiferi.*[8,9,10] The designation "golden staphylococci" refers to *Staphylococcus* species that possess pathogenic potential and share the common phenotypic trait of forming golden or yellowish colonies when cultured on conventional media.[11] This characteristic pigmentation primarily results from the production of carotenoid pigments, notably staphyloxanthin, a yellow-gold carotenoid. Beyond imparting distinctive colony coloration, staphyloxanthin plays a crucial role in bacterial virulence by acting as an antioxidant that neutralizes reactive oxygen species, thereby protecting bacterial cells from oxidative stress.[12,13] This antioxidant capacity significantly enhances microbial survival within the host and contributes to the maintenance of pathogenicity.

Historically, the identification and differentiation of golden staphylococcal species have posed significant challenges due to their high phenotypic similarity, particularly among those producing the enzyme coagulase, a traditional diagnostic marker associated with pathogenic *Staphylococcus* species.[11] In this context, the *Staphylococcus intermedius* group, comprising *Staphylococcus intermedius*, *Staphylococcus pseudintermedius*, *Staphylococcus delphini*, and *Staphylococcus schleiferi*, was initially regarded as a cluster of exclusively veterinary pathogens. These species were frequently misidentified as *Staphylococcus aureus* due to overlapping phenotypic features, including golden pigment production and coagulase-positive activity.[14,15,16] Recent advances in molecular techniques, particularly whole-genome sequencing and phylogenetic analyses, have proven essential for accurate species identification. These approaches have not only revealed distinct evolutionary lineages but also uncovered host-specific genetic adaptations that facilitate efficient colonization of animal tissues and organs.[16,17,18]

In cats, the nasal cavity serves as a favorable ecological niche for colonization by *Staphylococcus* species, often in the absence of overt clinical signs. Nevertheless, such asymptomatic persistence should not be underestimated, as it represents a potential reservoir of pathogens capable of both zoonotic and retrozoonotic transmission.[10,19,20,21,22,23,24,25] Recent studies indicate that domestic cats can harbor golden staphylococcal strains with genetic profiles closely resembling those found in humans, including strains carrying genes encoding toxins and adhesion factors that enhance virulence.[23,24,26]

The associated risks are bidirectional. In cats, infection with golden staphylococci may manifest as dermatitis, otitis, post-surgical wound infections, or, rarely, sepsis, particularly in animals with comorbidities or immunosuppression.[10,27,28] For humans, the primary concern lies in the potential acquisition of multidrug-resistant strains[24,26,29,30,31,32,33], which can render zoonotic infections clinically challenging to treat and lead to increased morbidity, hospitalization rates, and mortality. Moreover, antimicrobial resistance selection may be amplified by the indiscriminate use of antibiotics in veterinary medicine, often without coordination with human medical treatment and frequently lacking appropriate microbiological guidance for either species.

Given the pathogenic, zoonotic, and reverse-zoonotic potential of coagulase-negative staphylococci, as well as their role as reservoirs of antimicrobial resistance genes, this study aimed to determine the prevalence of golden-pigmented *Staphylococcus* strains, defined by their ability to produce golden colonies on culture media, among nasal isolates from cats attending a veterinary clinic in Bom Jesus do Norte, Province of Espírito Santo, Brazil, and to characterize their antimicrobial susceptibility profiles using the Kirby-Bauer disk diffusion method.

MATERIAL AND METHODS

This study was designed as a cross-sectional, descriptive, retrospective, and observational investigation, with a sample representative of the feline population attending a veterinary clinic located in the city of Bom Jesus do Norte, in the southern region of the Province of Espírito Santo, Brazil. Data and biological samples were collected between February and December 2024. Prior to initiating any procedures, informed consent was obtained from the animals' caregivers following a thorough

explanation of the study's objectives, methods, including sample collection, microbiological culture, antimicrobial susceptibility testing (antibiogram), and data analysis, and its academic purpose, with the intention of publishing the findings in a scientific article.

Inclusion criteria were: no antibiotic administration within the six months preceding sample collection and signed informed consent by the animal owners or legal guardians. The final sample consisted of 45 cats of various breeds, aged between 2 months and 8 years, originating from Bom Jesus do Norte and neighboring cities.

Nasal samples were collected using sterile swabs, which were sequentially inserted into both nostrils of each animal and gently rotated against the internal nasal mucosa. Immediately after collection, each swab was inoculated onto two Petri dishes containing mannitol salt agar and sheep blood agar, respectively. The streak plate technique was employed to ensure bacterial colony isolation. Plates were incubated in a bacteriological incubator at 37°C for 24 hours, with a subsequent reassessment at 48 hours to detect microbial growth.

In cases where bacterial growth occurred, pure colonies were selected for preparation of bacterial suspensions in sterile saline pre-warmed to 37°C and incubated for at least 15 minutes. Suspension density was visually standardized to 0.5 McFarland units (equivalent to approximately 1×10^8 colony-forming units per milliliter) using a densitometer. The standardized suspensions were then evenly spread onto the surface of Mueller-Hinton agar plates using sterile swabs.

Antimicrobial susceptibility testing was performed using the disk diffusion method (Kirby-Bauer) with the commercial CEFAR kit, strictly adhering to the current guidelines of the European Committee on Antimicrobial Susceptibility Testing.[34] Sterile paper disks impregnated with the following antimicrobial agents were placed on the agar surface: ampicillin, azithromycin, cefoxitin, ciprofloxacin, clindamycin, chloramphenicol, erythromycin, gentamicin, linezolid, oxacillin, penicillin, rifampicin, sulfamethoxazole-trimethoprim, tetracycline, and vancomycin. Plates were incubated at 37°C for 24 hours. During incubation, antimicrobials diffused radially through the agar, creating concentration gradients that inhibited bacterial growth around the disks when the organism was susceptible to the tested agent.

Initial bacterial identification was based on colony morphology, Gram staining, and complementary biochemical tests, including catalase, hemolysis on blood agar, mannitol fermentation, tube coagulase, and deoxyribonuclease production. Given the focus on coagulase-positive staphylococci, particularly *Staphylococcus aureus*, the reference strain *Staphylococcus aureus* ATCC 25923 was used as a quality control in antimicrobial susceptibility assays.

Following incubation, inhibition zone diameters were measured in millimeters using a calibrated ruler under reflected light against a black background, in accordance with EUCAST recommendations. Results were interpreted using the latest clinical breakpoints, classifying isolates as resistant (R), susceptible (S), or susceptible with increased exposure (I), according to EUCAST criteria. This methodological approach enabled precise phenotypic characterization of the *Staphylococcus* spp. antimicrobial resistance patterns in the evaluated cats.

After interpretation of the antibiograms, results were compiled into a Microsoft Excel 2021 spreadsheet and subjected to statistical analysis using Posit RStudio software version 3.6.0.

RESULTS

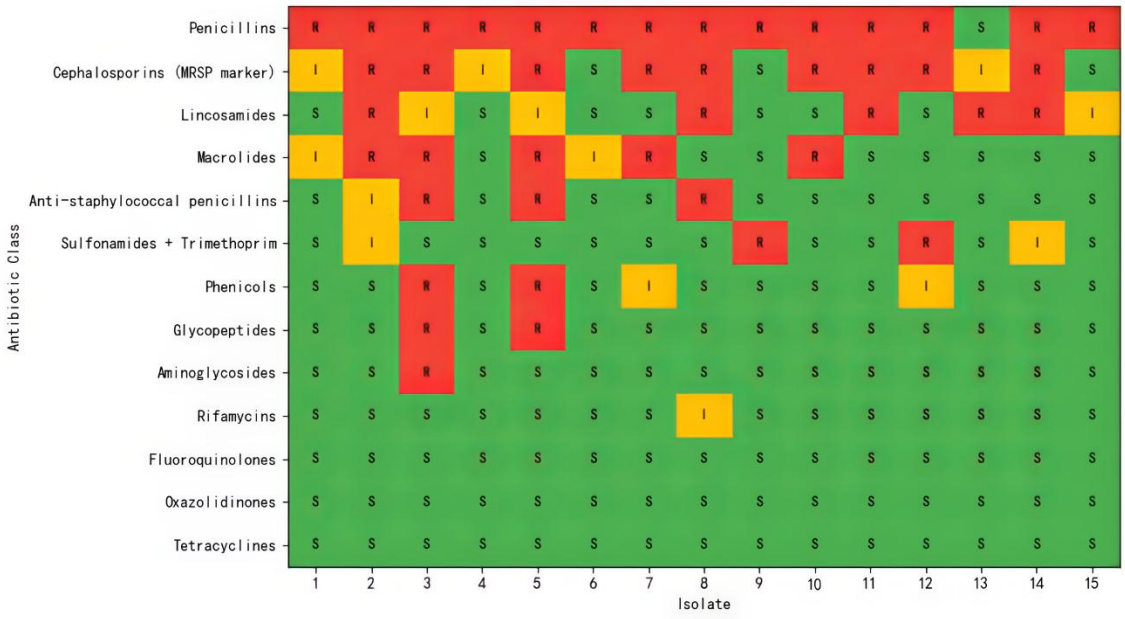
Among the 45 cats examined in this study, 15 yielded samples positive for coagulase-positive staphylococci producing golden-pigmented colonies, corresponding to a prevalence of 33.3%. Antibiotic resistance profiles exhibited considerable variability across isolates. A Venn diagram was designed to illustrate the relationships observed among the different antimicrobial agents, facilitating a more comprehensive analysis.

Table 1: Antibiotic susceptibility profile for each positive isolate of coagulase-positive *Staphylococcus* spp. with golden-pigmented colonies obtained from the nasal mucosa of cats treated at a veterinary clinic in Bom Jesus do Norte, Espírito Santo, Brazil.

Antibiotic	Sample identification number														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Ampicillin	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R
Azithromycin	I	S	R	S	R	S	R	S	S	R	S	S	S	S	S
Cefoxitin	I	R	R	I	R	S	R	R	S	R	R	R	I	R	S
Ciprofloxacin	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Clindamycin	S	R	I	S	I	S	S	R	S	S	R	S	R	R	I
Chloramphenicol	S	S	R	S	R	S	I	S	S	S	S	I	S	S	S
Erythromycin	S	R	R	S	S	I	S	S	S	S	S	S	S	S	S
Gentamicin	S	S	R	S	S	S	S	S	S	S	S	S	S	S	S
Linezolid	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Oxacillin	S	I	R	S	R	S	S	R	S	S	S	S	S	S	S

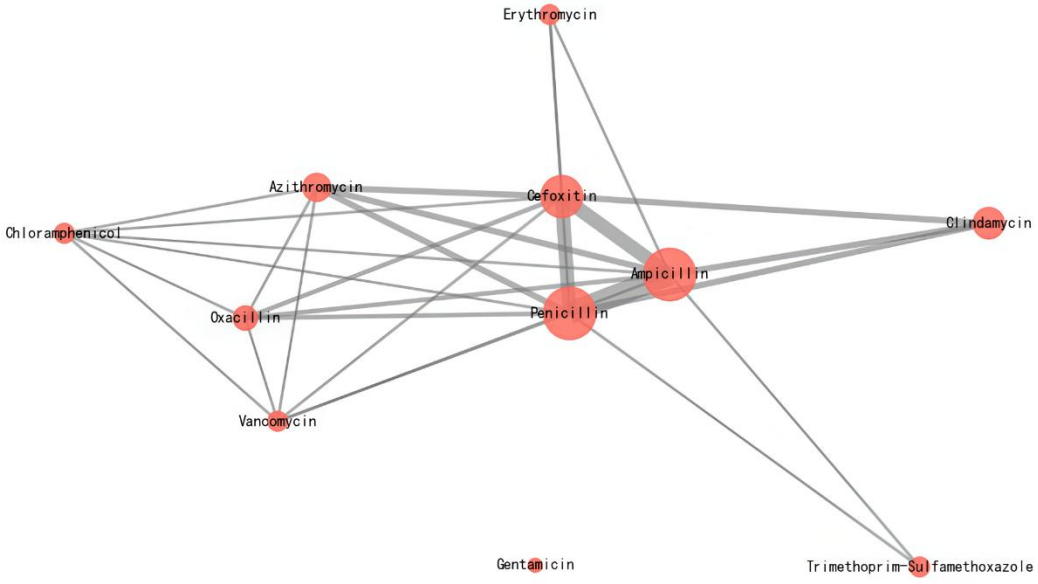
Penicillin	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R
Rifampicin	S	S	S	S	S	S	S	I	S	S	S	S	S	S	S
Trimethoprim–sulfamethoxazole	S	I	S	S	S	S	S	S	R	S	S	R	S	I	S
Tetracycline	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Vancomycin	S	S	R	S	R	S	S	S	S	S	S	S	S	S	S

** S = Sensible; I = Sensible, increased exposure; R = Resistant*



Graph 1: Heatmap of antimicrobial susceptibility profiles by antibiotic class, based on positive isolates of coagulase-positive *Staphylococcus* spp. with golden-pigmented colonies recovered from the nasal mucosa of cats attended at a veterinary clinic in Bom Jesus do Norte, Espírito Santo, Brazil.

** S = Sensible; I = Sensible, increased exposure; R = Resistant*



Graph 2: Venn diagram illustrating the relationships of resistance profiles for different antimicrobial agents based on positive isolates of coagulase-positive *Staphylococcus* spp. with golden-pigmented colonies recovered from the nasal mucosa of cats attended at a veterinary clinic in Bom Jesus do Norte, Espírito Santo, Brazil.

DISCUSSION

Numerous studies indicate that cats exhibit a lower prevalence of nasal colonization by golden staphylococci compared to dogs[35,36,37], which may be attributed to differences in nasal anatomy and potentially in grooming behaviors that influence microbial colonization. Physiological distinctions, such as obligate nasal breathing resulting from the close apposition of the epiglottis to the soft palate, also play a role in colonization dynamics. This anatomical feature affects airflow patterns and, consequently, the distribution and persistence of microbial communities in the nasopharynx. Such anatomical and physiological characteristics directly shape the nasal microbiota by creating distinct microenvironments within the nasal cavity that are influenced by airflow dynamics.[35,36,38] However, in dogs from Bom Jesus do Norte and surrounding areas, the prevalence of golden staphylococci in the nares was reported at 31.9%[39], a rate strikingly close to that observed in cats in our study, contrasting with the typical divergence in colonization rates between dogs and cats reported elsewhere globally.

According to several authors, the nasopharyngeal microbiome of healthy cats plays a critical role in respiratory health by acting as a barrier against pathogenic colonization and positively modulating host immune responses.[40,41,42] In vitro studies have demonstrated that *Staphylococcus felis*, a coagulase-negative species predominant in the feline nasopharynx, competitively inhibits the growth of methicillin-resistant *Staphylococcus pseudintermedius*.[43,44] This finding suggests that the biochemical mechanisms underlying *Staphylococcus felis*-mediated competitive exclusion may limit nasal colonization by *Staphylococcus pseudintermedius* in cats, contributing to the low prevalence of this species in the feline nasal microbiota. However, this mechanism appears ineffective against *Staphylococcus aureus*, a species that has evolved efficient strategies to colonize the nasal passages of diverse mammalian hosts, overcoming host defense mechanisms.[45,46] In this context, it is likely that among golden staphylococci isolated from the nasal cavities of cats, *Staphylococcus aureus* is the most prevalent species, and that the risks of zoonotic and reverse-zoonotic transmission are more significant than those observed between dogs and humans, owing to this bacterium's broad host tropism and capacity to colonize the nasopharynx across species.

Multiple studies confirm a higher prevalence of *Staphylococcus aureus* compared to other coagulase-positive *Staphylococcus* species as nasal colonizers in cats, along with a considerably lower incidence of *Staphylococcus pseudintermedius* colonization.[35,36,37,47] Bierowiec et al.[38] observed that *Staphylococcus aureus* isolates were recovered exclusively from healthy cats, whereas clinically ill cats predominantly harbored *Staphylococcus pseudintermedius* as the primary coagulase-positive staphylococcal species. The prevalence of *Staphylococcus aureus* colonization in cats varies significantly between pet and stray populations. A study conducted from January 2013 to November 2014 involving 150 cats found that 19.17% of pet cats were colonized by *Staphylococcus aureus*, compared to only 8.3% of stray cats.[38] Although phenotypic tests were used for initial characterization in our study, the absence of molecular identification precludes definitive species assignment. Future investigations should incorporate molecular methods to clarify whether the high prevalence of methicillin-resistant and multidrug-resistant strains reflects colonization by *Staphylococcus pseudintermedius*, *Staphylococcus schleiferi* or *Staphylococcus aureus*.

Antimicrobial susceptibility profiles of golden-pigmented *Staphylococcus* spp. vary considerably across geographic regions, likely influenced by climate, human and feline population density, and regional veterinary practices, including antibiotic prescribing patterns. However, these factors do not exclude underlying biological or ecological determinants that warrant further investigation to elucidate drivers of prevalence rates, species distribution of nasal *Staphylococcus* in cats, and resistance patterns not directly linked to veterinary antibiotic use.[48,49] In a study of dogs and cats in Rio de Janeiro, Pereira et al.[50] reported high resistance rates to penicillin and ampicillin, notably elevated resistance to ceftriaxone, oxacillin resistance in 37% of isolates, and low resistance to carbapenems and linezolid. These resistance profiles closely resemble those observed in cats from Bom Jesus do Norte and surrounding areas. Silva et al.[49] reported that 35% of *Staphylococcus pseudintermedius* isolates from cats were multidrug-resistant, with 20% of those being methicillin-resistant. Surprisingly, *Staphylococcus aureus* strains isolated in that study showed no multidrug resistance. Although our study did not identify the specific *Staphylococcus* species colonizing the feline nares, it is evident that the proportion of methicillin-resistant coagulase-positive *Staphylococcus* spp. in the cat population of Bom Jesus do Norte and neighboring municipalities exceeds the rates reported by Silva et al.[49] in the Province of Rio Grande do Sul.

Hierarchical clustering embedded in the heatmap revealed two distinct groups: one composed of methicillin-resistant, multidrug-resistant isolates, and another consisting of strains susceptible or resistant only to natural penicillins, probably due to β-lactamase production. Concurrently, an analysis of antibiotic co-resistance based on susceptibility profiles of 15 *Staphylococcus* spp. isolates from the feline nasopharynx identified verifiable patterns depicted in the Venn diagram. In this network analysis, nodes represent antibiotics to which at least one isolate exhibited resistance, with node size proportional to the total number of resistant isolates. Edges connect pairs of antibiotics for which concurrent resistance occurred in two or more isolates, with edge thickness directly

proportional to the frequency of co-resistance. Results revealed a central co-resistance core formed by ampicillin and penicillin, with identical resistance profiles in 14 of the 15 isolates. This core was strongly linked to cefoxitin (9 isolates co-resistant to both β-lactams) and less associated with azithromycin, clindamycin, chloramphenicol, and vancomycin (2 to 4 isolates resistant to each pair). This pattern is characteristic of methicillin-resistant *Staphylococcus*. Cefoxitin, used as a phenotypic surrogate for methicillin resistance, served as the network hub, connecting to ampicillin, penicillin, azithromycin, clindamycin, chloramphenicol, and vancomycin. This centrality underscores the tendency of methicillin-resistant *Staphylococcus* spp. isolates to acquire multiple resistance mechanisms, likely mediated by mobile genetic elements such as plasmids or resistance genomic islands. The high proportion of isolates with intermediate susceptibility to chloramphenicol (30.4%) and oxacillin suggests possible selection of emerging resistance mechanisms that warrant ongoing surveillance, as they may progress to full resistance without appropriate antimicrobial stewardship.[30] The high likelihood that cats harbor strains acquired through reverse zoonosis further underscores the need for coordinated, One Health–oriented antimicrobial prescribing practices in both human and veterinary medicine. The exclusion of animals with recent antimicrobial exposure strengthens the study's internal validity but may limit generalizability to populations with high antimicrobial exposure, where methicillin-resistant strain prevalence could be even higher.

Phylogenetic studies provide strong evidence that methicillin-resistant *Staphylococcus aureus* strains circulate bidirectionally between cats and their human caregivers.[23,26,51,52,53,54,55] Compared to dogs, cats have demonstrated a slightly higher baseline prevalence as MRSA reservoirs.[54] Given that *Staphylococcus aureus* is the most abundant golden staphylococcal species in both cats and humans, these animals represent significant reservoirs of antibiotic-resistant *Staphylococcus aureus*. A meta-analysis by Abdullahi et al.[56] estimated the global prevalence of MRSA in cats at approximately 0.5%. In contrast, our study found a methicillin resistance prevalence, indicated by cefoxitin resistance, of 66.6% among cats carrying golden-pigmented *Staphylococcus* spp., and 22.2% across the entire sampled feline population. Although the sample size is modest, this finding strongly suggests that methicillin-resistant *Staphylococcus* spp. constitute a substantial proportion of nasal colonizers in cats from Bom Jesus do Norte and adjacent municipalities.

Although azithromycin and erythromycin belong to the macrolide class, co-resistance was observed in only one isolate. Moreover, azithromycin showed no direct linkage to clindamycin in the resistance network, despite frequent co-occurrence in susceptibility profiles.[57,58] This disconnection suggests that, in this isolate collection, resistance genes for macrolides and lincosamides may reside on distinct genetic elements.

Antibiotics such as gentamicin, sulfamethoxazole–trimethoprim, and vancomycin occupied peripheral positions in the Venn diagram. Gentamicin resistance occurred in a single isolate, which was also multidrug-resistant. The high resistance rate to sulfamethoxazole–trimethoprim may reflect its preferential use in veterinary prescriptions for the local feline population. Vancomycin resistance, observed in two isolates, coincided with β-lactam resistance, suggesting multidrug-resistant strains of possible reverse-zoonotic origin or those that have horizontally acquired resistance genes, particularly notable given that vancomycin is reserved as a last-line therapeutic agent in human medicine and is not used in veterinary practice. Although beyond the scope of our methodological approach, phenotypic vancomycin resistance warrants confirmation via molecular testing.

Ciprofloxacin, tetracycline, and rifampicin did not appear in the Venn diagram, as no isolate exhibited resistance to these agents. These antibiotics therefore represent reliable therapeutic options for managing staphylococcal infections in cats, especially in suspected methicillin-resistant cases. Similarly, no resistance to linezolid was observed; however, its high cost and restricted veterinary use limit its practicality for treating opportunistic infections in cats.

CONCLUSIONS

This study demonstrates that one third of cats attending a veterinary clinic in Bom Jesus do Norte, in the southern region of the Province of Espírito Santo, Brazil, are asymptomatically colonized in the nasal cavity by coagulase-positive *Staphylococcus* spp. producing golden-pigmented colonies, revealing a notably high carriage rate of these bacteria among clinically healthy animals. Of these isolates, 66.6% exhibited resistance to cefoxitin, a well-established phenotypic marker of methicillin resistance, and displayed multidrug-resistant profiles. This finding is especially concerning, as it suggests the circulation of highly transmissible resistant strains within households where close and prolonged contact between cats and humans is routine.

Antimicrobial susceptibility profiling further uncovered distinct co resistance patterns, with a core cluster of β lactam resistance (ampicillin and penicillin) consistently associated with resistance to macrolides, lincosamides, chloramphenicol, and, to a lesser extent, vancomycin, strongly indicative of multidrug resistant and probable retrozoonotic clonal lineages. Conversely, ciprofloxacin, linezolid, tetracycline, and rifampicin retained complete in vitro efficacy, thereby representing reliable therapeutic alternatives for managing staphylococcal infections in cats, particularly in cases suspected of multidrug resistance or opportunistic disease.

Although the study has limitations, notably the lack of molecular species identification and a modest sample size, the findings collectively reinforce the critical need for judicious antimicrobial use in veterinary practice and for strengthened One Health surveillance integrating human and animal health sectors. The high prevalence of methicillin resistant staphylococci in domestic cats constitutes not only a clinical risk for the animals themselves but also a potential zoonotic and reverse zoonotic reservoir of antimicrobial resistance genes, with direct public health implications. Future investigations should employ molecular typing methods to definitively identify circulating strains and assess their relatedness to human isolates, thereby strengthening the epidemiological foundation for coordinated and sustainable interventions. Ultimately, consolidating research data, enhancing integrated surveillance, and promoting transparent data sharing are essential steps toward effective, systemic, and globally informed policies to combat the growing challenge of antimicrobial resistance.

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