BMC Veterinary Research

RESEARCH

Open Access

Genotypic profile of *Staphylococcus* spp., *Enterococcus* spp., and *E. coli* colonizing dogs, surgeons, and environment during the intraoperative period: a cross-sectional study in a veterinary teaching hospital in Brazil

Mareliza Possa de Menezes[1], Marita Vedovelli Cardozo[2], Natália Pereira[2], Mariana Bugov[1], Newton Valerio Verbisck[3], Vanessa Castro[4], Alessandra Figueiredo de Castro Nassar[4] and Paola Castro Moraes[1*]

Abstract

Aims This prospective cross-sectional study aimed to determine the occurrence of resistance genes and genetic diversity in *Staphylococcus* spp., *Enterococcus* spp., and *Escherichia coli* isolated from dogs' superficial surgical site (SS), surgeons' hands, and the operating room (OR) during the intraoperative period.

Methods Thirty dogs undergoing clean/clean-contaminated (G1, $n=20$) and contaminated surgeries (G2, $n=10$), along with eight surgeons, were included in the study. Specimens were collected using sterile swabs, transported in 0.1% peptone salt solution, and spread onto blood agar. Environmental samples were collected through passive exposure using BHI agar plates. Seventy-five isolates were selected and classified using MALDI-TOF MS. Resistance genes were screened via PCR: *tet(M)*, *ermA*, *aacA-aphD*, *blaZ*, *mecA*, $bla_{\text{TEM-1}}$, bla_{SHV}, $bla_{\text{SHV-1}}$, $bla_{\text{CTX-M-1, 3 e 15}}$, $bla_{\text{CTX-M-2}}$, $bla_{\text{CMY-2}}$, mcr_1, mcr_2, mcr_3, mcr_4, and *ndm*. Genetic diversity was assessed through PFGE analysis using *Sma*I and *Xba*I restriction enzymes, with clustering performed by the UPGMA method. The chi-square test compared the frequency of resistance gene detected.

Results *Staphylococcus pseudintermedius* (83.33%), *Enterococcus* spp. (52.63%), and *E. coli* (62.50%) were more frequently isolated from dogs' skin, while coagulase-negative staphylococci (CoNS; 62.50%) were more frequent in the OR. Resistance genes detected in *Staphylococcus* spp. included *blaZ* (79.17%), *mecA* (43.75%), *tet(M)* (41.67%), and *aacA-aphD* (25%). Among *Enterococcus* spp., *tet(M)* (78.95%) and *blaZ* (10.53%) were identified. *S. pseudintermedius* harbored *tet(M)* and *aacA-aphD* genes more frequently than CoNS. No *E. coli* isolates tested positive for the investigated genes. Twenty-four PFGE banding patterns were observed in CoNS (24/24), 15 in *S. pseudintermedius* (15/24), 4 in *E. coli* (4/8), and 7 in *Enterococcus spp.* (7/19). Genetically related *S. pseudintermedius* and *E. coli* were obtained

*Correspondence:
Paola Castro Moraes
paola.moraes@unesp.br
Full list of author information is available at the end of the article

from SS and OR in G2. Seven indistinguishable *Enterococcus* spp. were identified across different procedures and patients.

Conclusion Our study revealed high rates of methicillin-resistant *Staphylococcus* spp. and tetracycline-resistant *Enterococcus* spp. colonizing the environment in a veterinary teaching hospital in Brazil. PFGE analysis indicated a high diversity of CoNS and Enterococcus spp. Genetically related strains in *S. pseudintermedius*, *Enterococcus* spp., and *E. coli* emphasize the importance of effective infection control policies to minimize the spread of resistant bacteria.

Keywords Epidemiological surveillance, Genetic diversity, Methicillin-resistant *Staphylococcus* spp., Multidrug-resistant bacteria, Tetracycline-resistant *Enterococcus* spp.

Background

Infections caused by multidrug-resistant (MDR) organism currently represent one of the most significant global challenges within the context of One Health, owing to their capacity to increase morbidity, mortality, and healthcare-associated costs [1]. Infections caused by bacteria classified as MDR are challenging because these bacteria exhibit resistance to at least one drug from three or more antimicrobial classes, severely limiting treatment options [2].

The hospital environment is reported as the main risk factor for acquiring resistant pathogens during hospitalization, both in human and veterinary settings. Community reservoirs of MDR bacteria could contribute to the heightened prevalence of these strains in the Intensive Care Unit (ICU), thereby compromising patient treatment and outcomes [3, 4].

Methicillin-resistant *Staphylococcus* spp. (MRS), Vancomycin-resistant *Enterococcus* spp. (VRE), and Extended-Spectrum β-Lactamase (ESBL)-producing Enterobacterales, such as *Klebsiella pneumoniae*, *Escherichia coli*, and *Proteus mirabilis*, are among the most common pathogens causing infections in critically ill veterinary patients [4, 5].

Although epidemiological data in Brazil's veterinary context is lacking, several studies have already reported a high prevalence of these pathogens colonizing patients or causing infections, especially MRS and ESBL-producing organisms [6–13]. Additionally, worldwide studies are also documenting the colonization of patients, healthcare professionals, and the environment within veterinary hospital settings by MDR bacteria [6, 11, 14].

This carriage could contribute to the development of surgical site infections (SSI), as well as to the dissemination of these strains among humans and dogs [4, 15]. Some studies have reported genetically related strains isolated from humans, veterinary patients, and the environment [16, 17]. In this way, molecular epidemiology appears as an important tool to better screen and elucidate the reservoirs and transmission dynamics of MDR bacteria in veterinary settings, aiming to develop strategies to overcome these challenges.

With this proposal, this cross-sectional epidemiological study aimed to determine the occurrence of resistance genes and the banding patterns in pulsed-field gel electrophoresis (PFGE) of *Staphylococcus* spp., *Enterococcus* spp., and *E. coli* isolated from dog's superficial surgical site (SS), surgeons' hands, and the operation room (OR) during the intraoperative period of clean/clean-contaminated, and contaminated surgeries in a veterinary teaching hospital located in the southeast of Brazil.

Results

Genotypic resistance

Among the bacterial species analyzed, within each species, *S. pseudintermedius* (83.33% [20/24]), *E. coli* (62.50% [5/8]), and *Enterococcus* spp. (52.63% [10/19]) were more frequently isolated from dogs' skin, while coagulase-negative staphylococci (CoNS; 62.50% [15/24]), were more frequent in the OR environment. Figure 1 A summarizes the bacteria isolated by the source of collection.

Regarding the staphylococcal species (Fig. 1B), the most frequent was *S. pseudintermedius* (50% [24/48]), followed by *S. haemolyticus* (19% [9/48]), and *S. epidermidis* (15% [7/48]). Among enterococcal species, *Enterococcus faecium* (89.47% [17/19]) was the most frequent, followed by *E. faecalis* (10.53% [2/19]).

When comparing the occurrence between the groups, *S. pseudintermedius* were more frequent in G2 (70% [21/30]) than in G1 (16.67% [3/18]), whereas CoNS were more frequent in G1 (83.33% [15/18]) than in G2 (30% [9/30]). Additionally, *Enterococcus* spp. were more frequent in G1 (68.42% [13/19]), whereas *E. coli* isolates were only identified in G2 (Fig. 2). Two out of 10 dogs in G2 (20%) developed SSI within 30 days after the intervention. These infections were caused by mixed species involving *S. pseudintermedius*, *Proteus mirabilis*, and *Pseudomonas aeruginosa* (P2), as well *S. pseudintermedius*, and *Enterobacter* spp. (P10). No patients in G1 presented with SSI within 30 days after the procedure.

In terms of the presence of resistance genes, *blaZ* was the most frequently detected overall. No statistically significant difference was found in the frequency of

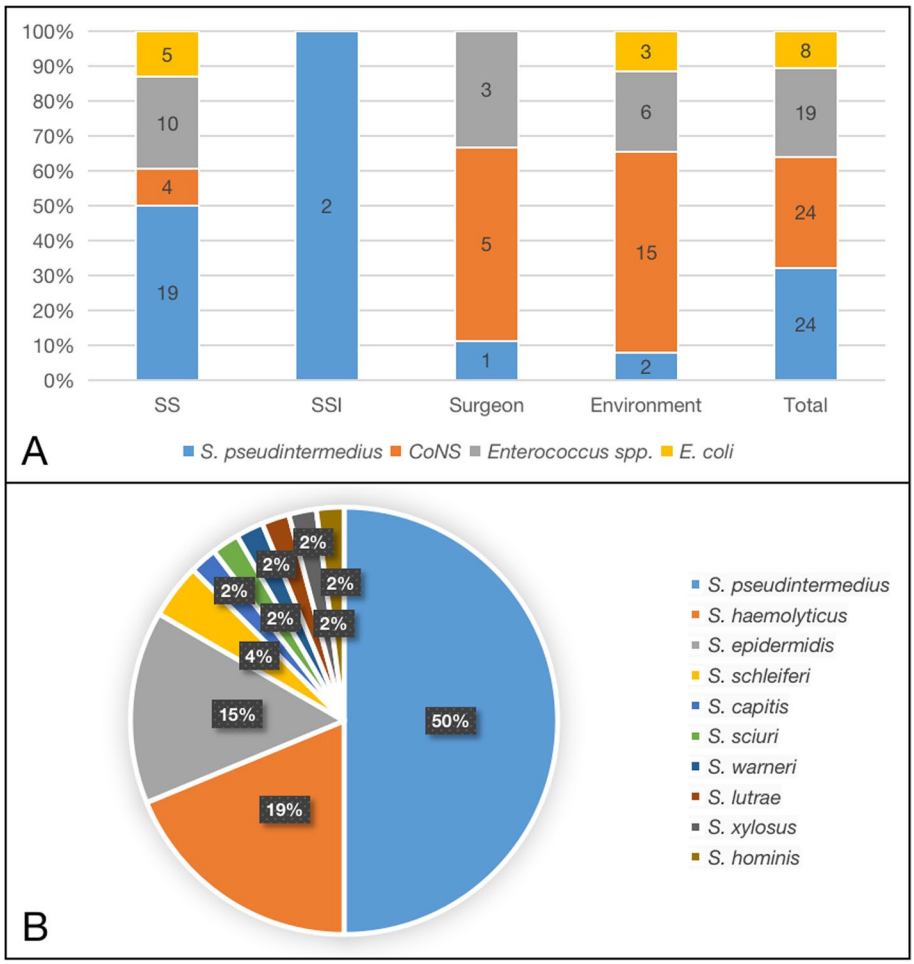

Fig. 1 Percentage distribution of overall bacterial species (**A**) and within *Staphylococcus* spp. (**B**) isolated per source of collection during the intraoperative period. Abbreviations: coagulase-negative staphylococci (CoNS); dogs' superficial surgical site (SS); surgical site infection (SSI)

resistance genes (FR) between isolates obtained from different sources ($p > 0.05$), as shown in Fig. 3A and Table 1. Among *Enterococcus* spp., *tet(M)* (78.95% [15/19]), was the most frequently detected, followed by *blaZ* (10.53% [2/19]). None of the isolates tested positive for *ermA*. No screened resistance genes were found in the *E. coli* isolates.

Among the staphylococcal isolates (Fig. 3B; Table 2), *blaZ* had the highest occurrence (79.17% [38/48]), followed by *mecA* (43.75% [21/48]), *tet(M)* (41.67% [20/48]), and *aacA-aphD* (25% [12/48]). CoNS (50% [12/24]) exhibited a higher frequency of *mecA*-positive isolates compared to *S. pseudintermedius* (37.50% [9/24]), although no statistical difference was observed between the rates ($p = 0.5606$). However, for the *tet(M)* and *aacA-aphD* genes, *S. pseudintermedius* showed a higher proportion ($p = 0.0404$ and $p = 0.0196$, respectively) of positive isolates (58.33% [14/24] and 41.67% [10/24], respectively) compared to CoNS (25% [6/24] and 8.33% [2/24], respectively). The phenotypic results for these isolates, obtained in a previous study [11], are summarized in Additional File 1.

Four out of 10 procedures (40%) whithin G2 and 7 out of 20 (35%) within G1 had at least one MRS isolated from any source.

Genetic diversity

Twenty-four PFGE banding patterns were observed in CoNS. *S. haemolyticus,* and *S. epidermidis* presented a Diversity Ratio (DR) of 100%. Fifteen PFGE banding patterns were observed in *S. pseudintermedius* (DR = 58.33% [14/24]), 4 in *E. coli* (DR = 50% [4/8]), and 7 in *Enterococcus faecium* and *E. faecalis* (DR = 36.84% [7/19]).

Regarding the staphylococcal species, CoNS isolates exhibited greater diversity compared to *S. pseudintermedius,* a coagulase-positive staphylococci (Fig. 4).

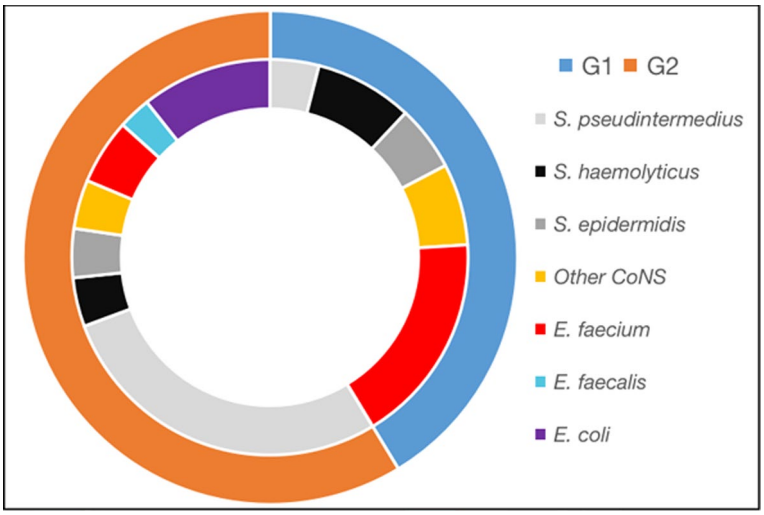

Fig. 2 Percentage distribution of overall bacterial species isolated during the intraoperative period, per type of surgery, in clean/contaminated (G1) and contaminated surgery (G2). *The outer ring differentiates between the two groups: clean/contaminated surgeries (G1, blue) and contaminated surgeries (G2, orange), while the inner ring represents the bacterial species isolated in G1 (right) and G2 (left). Abbreviations: clean/contaminated surgery (G1); contaminated surgery (G2); coagulase-negative staphylococci (CoNS)

Genetically related *S. pseudintermedius* isolated from different sources were obtained during two procedures (P2 and P10) in G2 (Fig. 4A). In contrast, none of the *S. haemolyticus,* and *S. epidermidis* isolates exhibited a high degree of similarity (≥ 90%) among the three distinct sources (Fig. 4B; 4C). Three isolates were obtained from SS (keys: 85, 87, and 90), 1 from OR (key: 82), and 1 causing SSI (key: 91) in P2. In P10, 1 isolate obtained from SS (key: 130) were genetically related to 1 isolate causing SSI (key: 137).

Four episode of genetically related *Enterococcus* spp. were identified across different procedures, and distinct patients. Isolates 42 and 61 (from P15 and P19); 102, 104, and 108 (from P3 and P4); and 9, 10, and 15 (from P5 and P6) were obtained from SS and exhibited high similarity (Fig. 5A). Another episode was found among eight isolates obtained from OR environment (11, 16, 34, and 66), SS (60, and 65), and surgeons' hands (98 and 99).

Despite the limited number of *E. coli* isolates obtained in this study, one episode of genetically related isolates was found (Fig. 5B). In P10, three isolates obtained from the environment (keys: 121, 122, and 123) were similar to one obtained from SS (key: 147).

Discussion

This cross-sectional epidemiological study reveals the presence of significant resistance genes identified in *Staphylococcus* spp., *Enterococcus* spp., and *E. coli*, along with their genotypic patterns.

S. pseudintermedius, *S. epidermidis*, and *S. haemolyticus* are common pathogens that colonize the skin and mucous membranes of dogs and humans, except for *S. pseudintermedius,* which is typically found in dogs but rarely in humans. They are oportunistic pathogens and a common cause of healthcare-associated infections, including SSIs [18, 19]. The colonization by MDR *Staphylococcus* spp. appears as one of the main risk factors for acquiring serious infections [5].

Only one (12.5% [1/8]) *S. pseudintermedius* isolate was obtained from surgeon's hands in this study; however it was not genetically related to other isolates found in the environment or dog's skin, suggesting human colonization by this strain. The close contact between humans and pets nowadays is increasing the prevalence of this species, particularly among veterinary workers [14, 16, 17, 20, 21].

Additionally, genetically related strains obtained from different sources were found among *S. pseudintermedius* isolated in two procedures in G2 (20% [2/10]), while in G1, there was no evidence of shared strains during a same procedure. This is likely due to contaminated surgeries presenting a higher microbial load in the surgical site, despite of not showing an infection. This high microbial content could also increase the possibility of fomites and environment contamination during the procedure, as well as increasing the SSI rates [22]. Thus, two out of 10 dogs in G2 (20%) developed SSI within 30 days after the intervention, caused by a mixed infection involving *S. pseudintermedius.* These

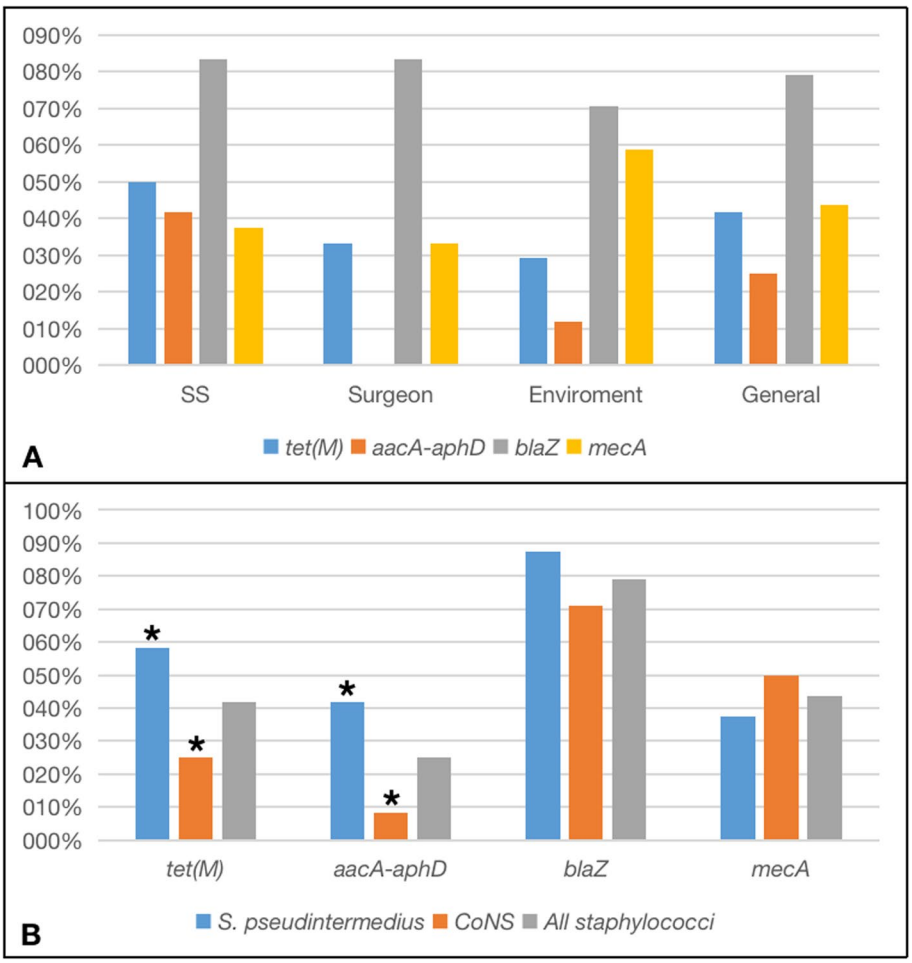

Fig. 3 Frequency of resistance genes detected in *Staphylococcus* sp., and *Enterococcus* sp. per collection source (A), and in *S. pseudintermedius* and coagulase-negative staphylococci (B) isolated from different sources during the intraoperative period in clean/clean-contaminated and contaminated surgery. *A statistical difference was found when comparing the rates of positive resistance genes among staphylococcal isolates using the chi-square test, with a significance threshold set at 0.05 and analysis conducted with R 4.3.3 software. Abbreviations: coagulase-negative staphylococci (CoNS); dogs' superficial surgical site (SS); surgical site infection (SSI)

Table 1 Frequency of *Staphylococcus* spp. isolates positive for selected resistance genes by source of collection

Source	*tet(M)*		*aacA-aphD*		*blaZ*		*mecA*	
	N	%	N	%	N	%	N	%
SS ($n=25$)	12	50,00%	10	41,67%	20	83,33%	9	37,50%
Surgeon ($n=6$)	2	33,33%	0	0,00%	5	83,33%	2	33,33%
Environment ($n=17$)	5	29,41%	2	11,76%	12	70,59%	10	58,82%

* Value significantly different ($p<0.05$) between groups compared by chi-square test, with a significance threshold set at 0.05 and analysis conducted with R 4.3.3 software

Abbreviations: dogs' superficial surgical site (SS)

isolates are similar to those obtained from the patients' skin during the intraoperative period (Fig. 4A).

Previous studies conducted in veterinary settings have already reported genetic relatedness in bacterial strains among dogs, personnel, and the hospital enviroment, identifying this as a risk factor for acquiring healthcare-associated infections [14, 23]. Interestingly, a high occurence of staphylococcal isolates in the present study harbored *blaZ* (79.17%), *mecA* (43.75%), *tet(M)* (41.67%), and *aacA-aphD* (25%), genes

Table 2 Frequency of isolates positive for selected resistance genes in *Staphylococcus pseudintermedius* and coagulase-negative staphylococci species

Species	*tet(M)*		*aacA-aphD*		*blaZ*		*mecA*	
	N	%	N	%	N	%	N	%
S. pseudintermedius (*n* = 24)	14	58,33%*	10	41,67%*	21	87,50%	9	37,50%
CoNS (*n* = 24)	6	25,00%*	2	8,33%*	17	70,83%	12	50,00%
p-value	**0,04042**		**0,01963**		0,2863		0,5606	

* A statistical difference was found when comparing the rates of positive resistance genes among staphylococcal isolates using the chi-square test, with a significance threshold set at 0.05 and analysis conducted with R 4.3.3 software. Significant differences are highlighted in bold

Abbreviations: coagulase-negative staphylococci (CoNS), dogs' superficial surgical site (SS), surgical site infection (SSI)

conferring resistance to penicillins, beta-lactams, tetracycline, and aminoglycosides drugs, respectively. These results, corroborate previous findings regarding phenotypic resistance published by the author's group. High rates of antimicrobial resistance to penicillin (80–100%), oxacillin (50%), cefoxitin (53.33–75.86%), tetracycline (65–73.33%), gentamicin (34.48–66.67%), and erythromycin (73.33–75.86%) in disk diffusion tests were observed [12]. However, despite the high rates of phenotypic erythromycin resistance found, no isolates were positive for the *ermA* gene in the current study. This finding highlights the need for further investigation into the resistance mechanism of these isolates [24]. Similar resistance rates were recently reported by Teixeira et al. (2023) [13], regarding aminoglycosides (84%), penicillin (76%), tetracycline (53.3%), and oxacillin resistance (24%) located in the southeast of Brazil.

Staphylococcus spp. strains harboring the *mecA* gene are generally multidrug-resistant, as this gene confers resistance to all drugs of the β-lactam class, with the exception of fifth-generation cephalosporins, and often correlates with resistance to other antimicrobial classes as well [25]. This behavior severely restricts treatment options and compromises patient outcomes. Additionally, tetracycline (*tet*), gentamicin (*aacA-aphD*) and erythromycin (*ermA*) could be important alternative choices to treat MRS infections, avoiding the use of antibiotics with high toxicity, such as rifampicin or chloramphenicol, or those antimicrobials considered critically important in human medicine, such as vancomycin, linezolid, and fluoroquinolones [26]. Hence, the high rates of *tet(M)* and *aacA-aphD,* in addition to *blaZ* and *mecA*, detected in our study are concerning.

These rates of MRS contrast with the low frequency (< 10%) of MRS colonization reported in companion animal practice, veterinary healthcare professionals, and veterinary settings in some countries, such as Austria [27], Bangladesh [28], Germany [29], Tanzania [30], and United States [23, 31]. However, they corroborate with the moderate or high occurrence (> 10%) observed in Brazil [6, 7, 10, 11, 13], Iran [32], Italy [33], Nigeria [34], Poland [35], Portugal [36], South Africa [37], and Switzerland [38] with occurrence rates ranging from 10 to 85.9%.

While the importance of *S. epidermidis* as a pathogen in human medicine is well-established, its significance in veterinary medicine remains unclear and potentially underestimated. However, there is increasing recognition of CoNS, mainly *S. epidermidis* and *S. haemolyticus*, as potential pathogens causing nosocomial infections in veterinary settings [7, 12, 38]. The present study showed a higher frequency of methicillin-resistance in CoNS (50%) isolates than in *S. pseudintermedius* (37.50%), a coagulase-positive staphylococci. Although no statistical significance was found, this finding corroborates with the previous phenotypic results published by the author's group [11], and with the findings reported by Adiguzel et al. (2022) [39].

The high frequency of staphylococcal isolates harboring *mecA* gene in the present study, as well as those frequently reported worldwide over the last two decades, underscore the exponential emergence of MRS globally. This trend is notable even in countries that traditionally report a low prevalence of these strains, highlighting the imperative for effective surveillance efforts aimed at devising strategies to mitigate this threat [40]. Therefore, meticulous attention to infection control policies is imperative to address the spread of these resistant strains and mitigate their impact on animal health.

Enterococcus spp. are opportunistic pathogens capable of surviving for months in the environment under adverse conditions. They can cause nosocomial infections, especially in immunocompromised patients [41]. While less prevalent compared to *Staphylococcus* spp. in causing infections in dogs, the incidence of *Enterococcus* spp. infections is increasing, with multidrug-resistant strains posing a significant concern in both human and canine critical care settings [42, 43]. Despite their lower

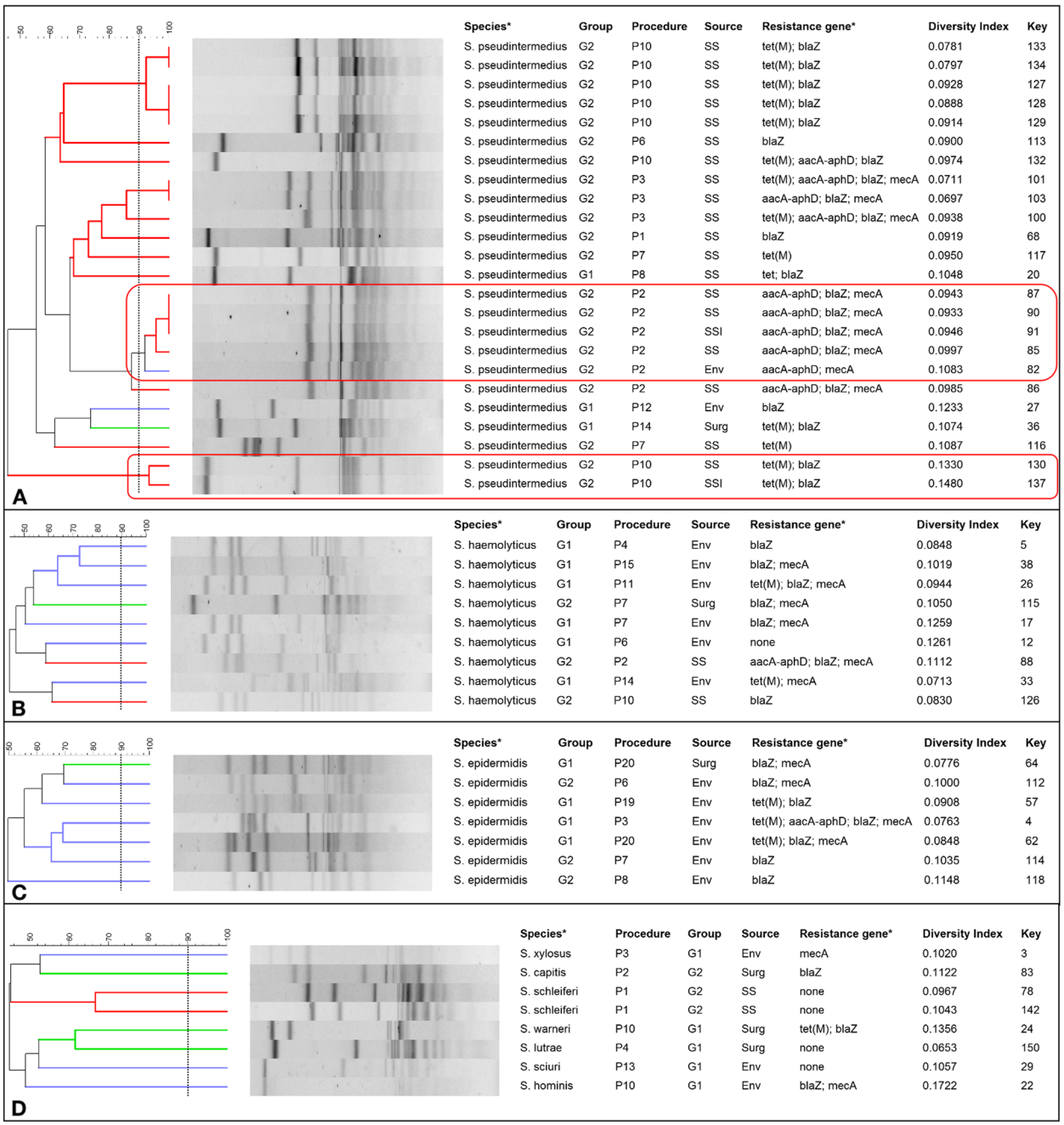

Fig. 4 Dendrogram produced by comparing banding patterns on PFGE of *Staphylococcus pseudintermedius* (**A**), *S. haemolyticus* (**B**), *S. epidermidis* (**C**), and other coagulase-negative staphylococci (**D**) species isolated from dogs' superficial surgical site (red); surgeons' hands (green), and operation room (blue). The dendrogram was generated using the UPGMA clustering method in BioNumerics 7.1 software. The Dice coefficient and optimization was set at 1%. PFGE similarity cutoff of 90% (dashed line) was applied. Red squares highlight the genetically related isolates. *Note: Species and genes names are not italicized due to software limitations (BioNumerics 7.1). Abbreviations: G1 = clean/clean contaminated surgery; G2: contaminated surgery; SS: dogs' superficial surgical site; SSI: surgical site infection; Env: environment; Surg: surgeon

prevalence, infections caused by enterococcal species are challenging to treat due to their diverse intrinsic antimicrobial resistance mechanisms and high level of acquired resistance, severely limiting treatment options [41, 44].

In the present study, *Enterococcus faecium* (89.47%) and *E. faecalis* (10.53%) were more frequently obtained from SS (52.63%) and from the OR environment (31.58% [6/19]). These isolates exhibited a different

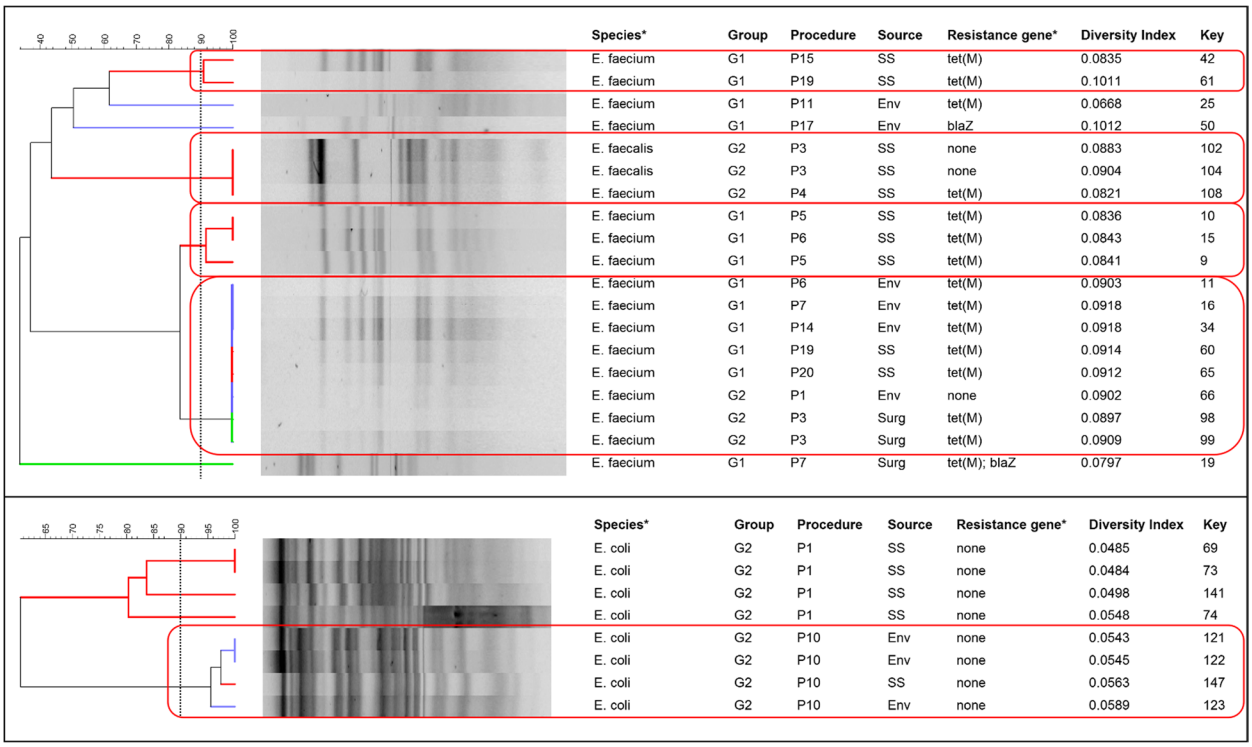

Fig. 5 Dendrogram produced by comparing banding patterns on PFGE of *Enterococcus* spp. (**A**) and *E. coli* (**B**) isolated from dogs' superficial surgical site (red); surgeons' hands (green), and operation room (blue). The dendrogram was generated using the UPGMA clustering method in BioNumerics 7.1 software. The Dice coefficient and optimization was set at 1%. PFGE similarity cutoff of 90% (dashed line) was applied. Red squares highlight the genetically related isolates. *Note: Species and genes names are not italicized due to software limitations (BioNumerics 7.1). Abbreviations: G1 = clean/clean contaminated surgery; G2: contaminated surgery; SS: dogs' superficial surgical site; SSI: surgical site infection; Env: environment; Surg: surgeon

diversity and similarity pattern (Fig. 5A) compared to *S. pseudintermedius* and CoNS, showing a lower FR than the other species. Interestingly, eight indistinguishable isolates by PFGE banding patterns were observed in five procedures in G1 (isolates key: 11, 16, 34, 60, and 65) and two procedures in G2 (isolates key: 66, 98, and 99). All but one of these isolates harbored the *tet(M)* gene. This observation likely indicates the persistence of a specific strain in the surgical environment and underscores the need for more appropriate cleaning and disinfection procedures tailored to this species. Chung et al. (2014) [45] previously reported a potential clonal cluster of antibiotic-resistant *Enterococcus* spp. isolated from both dogs and healthcare professionals in a veterinary hospital in Korea. Additionally, Ghosh et al. (2011) [46] documented a low FR of *E. faecium* in the feces of dogs in an ICU, along with strains genetically related among dogs, humans, and hospital outbreaks.

A high rate of enterococcal isolates harbored the tetracycline resistance gene (78.95%), which correlates with the phenotypic resistance reported previously (78.95%) by the author's group [11] and with findings reported by other authors [47–50]. However, the detection frequency of *blaZ* (10.53%), and *ermA* (0%) contrast with the phenotypic results for penicillin (68.42%), and erythromycin (84.24%) resistance for these specific isolates [11], and with those reported by other authors [47]. Several studies have reported an absence of a strong association between phenotypic and genotypic resistance to erythromycin. This highlights the urgent need for comprehensive molecular investigations of these species [49].

Finally, *E. coli* was isolated from two patients undergoing two different procedures. In one of these procedures, isolates were obtained from both the surgical site (isolate key: 147) and the environment (isolate key: 121, 122, and 123), exhibiting high similarity in PFGE banding patterns. Furthermore, all isolates tested negative for the investigated resistance genes, despite being phenotypically classified as MDR (Additional File 1). As the most often identified Gram-negative bacterium from animals, *E. coli* is responsible for a wide range of illnesses, including sepsis syndromes, gastrointestinal disorders, enterotoxemy, and SSI. Contaminated surgeries have a higher microbial load in the tissue and/or chronic inflammation,

making these sites more susceptible to contamination by environmental bacteria [4, 22]. Thus, it was expected that this bacterial species would be isolated only in G2.

The limited number of *E. coli* isolates in this study poses challenges for conducting a reliable analysis of genotypic antimicrobial resistance frequency. Additionally, the small sample size of procedures, dogs, and surgeons included in this study is another limitation, along with the restricted diversity of resistance genes screened. Despite these limitations, our study provides essential insights for epidemiological surveillance and suggests avenues for further cross-sectional and longitudinal research.

Conclusion

Our cross-sectional epidemiological study revealed high rates of MRS and tetracycline-resistant *Enterococcus* spp. colonizing the environment in a veterinary teaching hospital in the southeast of Brazil. PFGE analysis indicated a high diversity of CoNS among dogs, veterinarians, and the operating room. Genetically related strains were found in *S. pseudintermedius*, *Enterococcus* spp., and *E. coli* isolates, emphasizing the importance of effective infection control policies to minimize the spread of multidrug-resistant bacteria. Hospital environments and dogs can serve as sources of multidrug-resistant bacterial strains. Enhancing awareness and monitoring of these organisms in veterinary environments can aid in the prevention and control of infections. Continuous passive and active surveillance are crucial for achieving this goal.

Methods

Study design and population

A prospective cross-sectional epidemiological study was conducted at a veterinary teaching hospital in São Paulo state, Brazil. Seventy-five isolates were obtained from the superficial surgical site (SS) of 30 dogs that had not received antimicrobial therapy in the 72 h prior to collection, as well as from the operating room environment, and 8 veterinary surgeons in 30 different procedures. These dogs underwent either clean/clean-contaminated (G1 [n = 20]) or contaminated (G2 [$n = 10$]) surgeries.

The isolates were obtained from a previously published study conducted at the same veterinary teaching hospital during the year 2019 [11]. The owners of the dogs and the surgeons selected for this study provided informed consent for their participation.

Sample collection

In a previous study [11], specimens were collected using a dry, sterile cotton-tipped swab and transported in a sterile tube containing 0.1% peptone salt solution. For surgeons samples, swabs were rubbed in a circular motion from the wrists to the fingertips (both dorsal and palmar surfaces), with this procedure repeated three times for each finger. For dog's skin samples, the swab was also rubbed over the superficial surgical site (SS). The swabs were then spread onto 5% bovine blood agar (Oxoid, United Kingdom).

Environmental samples were collected via passive exposure using a Petri dish with Brain Heart Infusion agar (BHI; Oxoid, United Kingdom), which was left open on a 1-m-high support positioned near the surgery table during the procedure. All samples were incubated at 37 °C for 24 h under aerobic conditions. All morphologically distinct colonies from each sample were selected for further analysis.

Bacterial species identification

Each obtained colony was preserved in BHI broth with a 30% glycerol solution (1:1) at −80 °C and subsequently inoculated into BHI broth prior to each analysis.

Forty-eight *Staphylococcus* sp., 19 *Enterococcus* sp., and 8 *Escherichia coli* isolates, identified by Matrix-Assisted Laser Desorption Ionization Time-Of-Flight Mass Spectrometry (MALDI-TOF MS), were selected for this study. Sample preparation, data acquisition, and analysis were conducted as previously described by Sauer et al. (2008) [51] and Freiwald & Sauer (2009) [52]. The MALDI Biotyper 3.1 software from Bruker Daltonics was utilized to identify bacterial isolates based on their mass spectra obtained by an Autoflex III Smartbeam mass spectrometer. Standard Bruker interpretative criteria were applied; scores ≥ 2.0 were considered indicative of high reliability, while scores between 1.7 and 1.99 were considered indicative of moderate reliability. Isolates with a score < 1.7 were excluded from this study.

Resistance genes detection

DNA extraction

The DNA extraction of *Staphylococcus* spp. and *Enterococcus* spp. isolates was carried out following the method proposed by Bag et al. (2016) [53] and Moraes et al. (2022) [54] with some modifications. Briefly, aliquots of 1.5 mL of bacterial culture from each isolate were centrifuged at 8000 rpm for 5 min at 12 °C to obtain a sufficient cell pellet. The culture medium was discarded, and the bacterial pellet was resuspended in 700 µL of DNA extraction buffer [Tris–HCl 160 mM pH 8.0; EDTA 60 mM pH 8.0; NaCl 20 mM; SDS 0.5% (w/v)]. Cell lysis occurred at 65 °C for 40 min. Subsequently, 300 µL of 5 M potassium acetate solution was added to the solution, which, after homogenization, was kept on ice bathing for 30 min. Purification was performed using 600 µL of chloroform:isoamyl alcohol 24:1 (v/v) under

centrifugation at 12,000 rpm at 10 °C for 10 min. The clear supernatant was transferred to new tubes to which 1000 µL of chilled absolute ethanol was added. The solution was homogenized and kept in a freezer at −20 °C for 12 h for DNA precipitation. Subsequently, the tubes were centrifuged at 12,000 rpm at 10 °C for 17 min to obtain the DNA pellet. The supernatant was discarded, and the pellet was washed with 700 µL of 70% (v/v) ethanol, dried at 55 °C, and resuspended in 30–50 µL of TE buffer 10:1 (Tris–HCl 10 mM pH 8.0; EDTA 1 mM pH 8.0).

The DNA of Gram-negative bacteria was obtained using the boiling extraction method, as described by Keskimaki et al. (2001) [55].

PCR amplification

Resistance genes were detected through PCR amplification of selected genes. PCR conditions for detection of tested genes were carried out following previously described protocols provided in the Addtional File 2.

The genes *tet(M)* [56], *aacA-aphD* [56], *ermA* [56], *and blaZ* [57] were screened in *Staphylococcus* spp. and *Enterococcus* spp. isolates, while *mecA* [58] was exclusively targeted in *Staphylococcus* spp. For *E. coli,* the following genes were investigated: $bla_{\text{TEM-1}}$ [59], bla_{SHV} [60], $bla_{\text{SHV-1}}$ [59], $bla_{\text{CTX-M-1, 3 e 15}}$ [61], $bla_{\text{CTX-M-2}}$ [61], $bla_{\text{CMY-2}}$ [62], mcr_1 [63], mcr_2 [63], mcr_3 [63], mcr_4 [63], and *ndm* [64].

The PCR protocol followed the method described by CHINA et al. (1996) [65] with slight modifications. Briefly, a DNA aliquot (2 µL) was added to a mixture containing 0.4 µL of dNTP (2 mM), 2 µL of 10X buffer solution (200 mM Tris–HCl; 500 mM KCl; 20 mM $MgCl_2$ [pH 8.5]), 0.8 µL of $MgCl_2$ (25 mM), 0.2 µL of Taq DNA polymerase, and 1 µL of each primer (10 pmol), forward and reverse. The final volume of 20 µL was achieved with sterile MilliQ water. An aliquot of 5 µL of Gel Loading Dye Blue (0.25% bromophenol blue in 50% glycerol) was added to the PCR product. A molecular marker (100 pb or 1000 pb) was applied to a 1.5% agarose gel with SYBR® Safe DNA Gel Stain 10X (Thermo Fisher Scientific, Brazil). The eletrophoresis was runned in a Tris–Borate, EDTA buffer (120 V).

Bacterial genotyping

Strain genotyping was conducted using PFGE. Genomic DNA from all *Staphylococcus* spp. and *Enterococcus* spp. isolates was digested with *Sma*I, and from *E. coli* and *Salmonella* spp. with *Xba*I. Subsequently, they were separated by PFGE using the standardized Centers for Disease Control (CDC) protocol [66]. The pulse switch times for *E. coli* were 2.2 s initial time, 54.2 s final time, with a gradient of 6 V cm^{-1} and an angle of 120°, at 14 °C for 21 h; and for *Staphylococcus* spp. and *Enterococcus* spp. were 5.0 s initial time, 40.0 s final time, with a gradient of 6 V cm^{-1} and an angle of 120°, at 14 °C for 19 h. The universal size marker *Salmonella* serotype Braenderup H9812 were used on every gel [67].

Data analysis

Data generated were subjected to descriptive statistics using WPS Office spreadsheet© version 5.7.1 (Kingsoft Office Corporation, China) and expressed in percentages.

The frequency of resistance genes (FR) was calculated by WPS Office spreadsheet according to the formula:

$$FR(\%) = \left(\frac{\text{number of positive isolates for a specific gene}}{\text{total number of isolates tested for a specific gene}}\right) \text{x } 100$$

The chi-square test was applied using R 4.3.3 software© (R Foundation for Statistical Computing, Austria) to compare bacterial resistance rates between isolates obtained from different sources (within the same species) or among different species. A significance threshold of 0.05 was applied.

DNA fingerprints were analyzed using BioNumerics 7.1 software (Applied Maths NV, bioMérieux, Belgium), and the similarity of PFGE fragments obtained was compared using a Dice coefficient with a tolerance and optimization of 1%. The dendrogram was generated using the UPGMA clustering method. A PFGE similarity cutoff of 90% was applied to consider isolates as genetically related.

Diversity ratio (DR) was calculated by WPS Office spreadsheet according to the formula:

$$DR(\%) = \left(\frac{\text{number of PFGE banding patterns}}{\text{total number of isolates}}\right) \text{x } 100$$

Abbreviations

BHI	Brain heart infusion
CoNS	Coagulase-negative staphylococci
DR	Diversity ratio
ESBL	Extended-spectrum β-lactamase
FR	Frequency of resistance gene
G1	Clean/clean-contaminated surgery
G2	Contaminated surgery
ICU	Intensive care unit
MALDI-TOF MS	Matrix-assisted laser desorption ionization time-of-flight mass spectrometry
MDR	Multidrug-resistant
MRS	Methicillin-resistant *Staphylococcus* spp.
OR	Operation room
PFGE	Pulsed-field gel electrophoresis
SS	Superficial surgical site
VRE	Vancomycin-resistant *Enterococcus* spp

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12917-025-04611-4.

Additional file 1: Summary of phenotypic and genotypic resistance in strains isolated from dogs' surgical site, surgeons' hands, and operation room during the intraoperative period in clean/clean-contaminated and

contaminated surgery. Addtional Table 1. Summary of phenotypic and genotypic resistance in strains isolated from dogs' surgical site, surgeons' hands, and operation room during the intraoperative period in clean/clean-contaminated surgery. Addtional Table 2. Summary of phenotypic and genotypic resistance in strains isolated from dogs' surgical site, surgeons' hands, and operation room during the intraoperative period in contaminated surgery

Additional file 2: Resistance genes amplified by PCR in *Escherichia coli, Staphylococcus* spp., and *Enterococcus* isolated from dogs' surgical site, surgeons' hands, and operation room in a Veterinary Teaching Hospital in Brazil. Addtional Table 1. Resistance genes amplified by PCR in *Escherichia coli* isolated from dogs' surgical site, surgeons' hands, and operation room in a Veterinary Teaching Hospital in Brazil. Addtional Table 2. Resistance genes amplified by PCR in *Staphylococcus* spp., and *Enterococcus* spp. isolated from dogs' surgical site, surgeons' hands, and operation room in a Veterinary Teaching Hospital in Brazil.

Acknowledgements
We express our gratitude to Prof. Dr. Eliana Gertrudes de Macedo Lemos from the Department of Agricultural and Environmental Biotechnology at the School of Agricultural Sciences and Veterinary Medicine (UNESP) for providing access to Bionumerics 7.1® software. We also acknowledge São Paulo State University (UNESP) for its support of our study.

Authors' contributions
MVC, PCM, and MPM participated in the conceptualization, study design, and manuscript writing. MPM collected, processed, analyzed datas, and prepared Figs. 1–4. MPM, NP, and MB performed PCR and PFGE analysis. MPM, NVV, VC, and AFCN performed MALDI-TOF analysis. MPM, MVC, and NVV interpreted data. All authors read and approved the final manuscript.

Funding
This study was financed, in part, by São Paulo Research Foundation (FAPESP). Process number#2019/20585-0 and#2023/06904-0. And by the National Council for Scientific and Technological Development (CNPq), process number#307791/2021-1. The funding body played no role in the design of the study, data collection, analysis, interpretation, or in writing the manuscript.

Data availability
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
This study followed the recommendations of the Brazilian National Council for the Control of Animal Experimentation (CONCEA) and National Health Council (CNS), and was approved by the Institutional Ethics Committee in the Use of Animals (protocol number 5436/20), the Research Ethics Committee (CAAE: 07111419.4.0000.9029, and 39163720.5.0000.9029), and the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SisGen) (protocol nº A621D91). Dogs' owners and surgeons were informed of the methods and purpose of the study and provided written informed consent.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Author details
[1]Department of Clinic and Veterinary Surgery, School of Agricultural and Veterinarian Sciences, São Paulo State University (UNESP), Jaboticabal, São Paulo 14884-900, Brazil. [2]Department of Pathology, Reproduction, and One Health, School of Agricultural and Veterinarian Sciences, São Paulo State University (UNESP), Jaboticabal, São Paulo 14884-900, Brazil. [3]Embrapa Beef Cattle, Campo Grande, Mato Grosso do Sul, Brazil 79106-550. [4]Research and Development Center in Animal Health, General Bacteriology Laboratory, Biological Institute, São Paulo, São Paulo 04016-035, Brazil.

Received: 29 April 2024 Accepted: 19 February 2025
Published online: 06 March 2025

References

1. McEwen SA, Collignon PJ. Antimicrobial Resistance: a One Health Perspective. Microbiol Spectr. 2018;6:10.1128/microbiolspec.arba-0009-2017.
2. Magiorakos A-P, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect. 2012;18:268–81.
3. Hanczvikkel A, Tóth Á. Quantitative study about the role of environmental conditions in the survival capability of multidrug-resistant bacteria. J Infect Public Health. 2018;11:801–6.
4. Verwilghen D, Singh A. Fighting Surgical Site Infections in Small Animals. Are We Getting Anywhere? Veterinary Clinics of North America - Small Animal Practice. 2015;45:243–76.
5. Walther B, Tedin K, Lübke-Becker A. Multidrug-resistant opportunistic pathogens challenging veterinary infection control. Vet Microbiol. 2017;200:71–8.
6. de Menezes MP, Facin AC, Cardozo MV, Costa MT, Moraes PC. Evaluation of the Resistance Profile of Bacteria Obtained From Infected Sites of Dogs in a Veterinary Teaching Hospital in Brazil: A Retrospective Study. Top Companion Anim Med. 2021;42:100489.
7. Viegas FM, Santana JA, Silva BA, Xavier RGC, Bonisson CT, Câmara JLS, et al. Occurrence and characterization of methicillin-resistant Staphylococcus spp in diseased dogs in Brazil. PLoS One. 2022;17:e0269422.
8. Salgado-Caxito M, Moreno-Switt AI, Paes AC, Shiva C, Munita JM, Rivas L, et al. Higher prevalence of extended-spectrum cephalosporin-resistant enterobacterales in dogs attended for enteric viruses in brazil before and after treatment with cephalosporins. Antibiotics. 2021;10:1–13.
9. Salgado-Caxito M, Benavides JA, Munita JM, Rivas L, García P, Listoni FJP, et al. Risk factors associated with faecal carriage of extended-spectrum cephalosporin-resistant Escherichia coli among dogs in Southeast Brazil. Prev Vet Med. 2021;190:105316.
10. Teixeira IM, de Oliveira FE, de Araújo PB. Dogs as reservoir of methicillin resistant coagulase negative staphylococci strains – A possible neglected risk. Microb Pathog. 2019;135:103616.
11. Menezes MP, Borzi MM, Ruaro MA, Cardozo MV, Rabelo RC, Verbisck NV, et al. Multidrug-Resistant Bacteria Isolated From Surgical Site of Dogs, Surgeon's Hands and Operating Room in a Veterinary Teaching Hospital in Brazil. Top Companion Anim Med. 2022;49:100638.
12. Silva BA, do Amarante VS, Xavier RGC, Colombo SA, da Silva TF, Brenig B, et al. Characterization of ESBL/AmpC-producing extraintestinal Escherichia coli (ExPEC) in dogs treated at a veterinary hospital in Brazil. Res Vet Sci. 2024;166:105106.
13. Teixeira IM, de Moraes AY, Paletta ACC, Aguiar L, Guimarães L, da Silva IT, et al. Investigation of antimicrobial susceptibility and genetic diversity among Staphylococcus pseudintermedius isolated from dogs in Rio de Janeiro. Sci Rep. 2023;13:20219.
14. Feßler AT, Schuenemann R, Kadlec K, Hensel V, Brombach J, Murugaiyan J, et al. Methicillin-resistant Staphylococcus aureus (MRSA) and methicillin-resistant Staphylococcus pseudintermedius (MRSP) among employees and in the environment of a small animal hospital. Vet Microbiol. 2018;221:153–8.
15. Foschi D, Yakushkina A, Cammarata F, Lamperti G, Colombo F, Rimoldi S, et al. Surgical site infections caused by multi-drug resistant organisms: a case–control study in general surgery. Updates Surg. 2022;74:1763–71.
16. Lozano C, Rezusta A, Ferrer I, Pérez-Laguna V, Zarazaga M, Ruiz-Ripa L, et al. *Staphylococcus pseudintermedius* Human Infection Cases in Spain: Dog-to-Human Transmission. Vector-Borne and Zoonotic Diseases. 2017;17:268–70.
17. Jin M, Osman M, Green BA, Yang Y, Ahuja A, Lu Z, et al. Evidence for the transmission of antimicrobial resistant bacteria between humans and companion animals: A scoping review. One Health. 2023;17: 100593.

18. Chaudhary R, Thapa SK, Rana JC, Shah PK. Surgical Site Infections and Antimicrobial Resistance Pattern. J Nepal Health Res Counc. 2017;15:120–3.
19. Shoen HRC, Rose SJ, Ramsey SA, de Morais H, Bermudez LE, Analysis of Staphylococcus infections in a veterinary teaching hospital from,. to 2015. Comp Immunol Microbiol Infect Dis. 2012;2019:66.
20. Rodrigues AC, Belas A, Marques C, Cruz L, Gama LT, Pomba C. Risk Factors for Nasal Colonization by Methicillin-Resistant Staphylococci in Healthy Humans in Professional Daily Contact with Companion Animals in Portugal. Microb Drug Resist. 2018;24:434–46.
21. Moses IB, Santos FF, Gales AC. Human Colonization and Infection by Staphylococcus pseudintermedius: An Emerging and Underestimated Zoonotic Pathogen. Microorganisms. 2023;11:581.
22. Mangram AJ, Horan TC, Pearson ML, Silver LC, Jarvis WR. Guideline for Prevention of Surgical Site Infection, 1999. Centers for Disease Control and Prevention (CDC) Hospital Infection Control Practices Advisory Committee. Am J Infect Control. 1999;27:97–132; quiz 133–4; discussion 96.
23. Perkins AV, Sellon DC, Gay JM, Lofgren ET, Moore DA, Jones LP, et al. Prevalence of methicillin-resistant Staphylococcus pseudintermedius on hand-contact and animal-contact surfaces in companion animal community hospitals. Can Vet J. 2020;61:613–20.
24. Rasool Z, Noreen H, Anjum A, Rizvi A, Rabaan AA, Halwani MA, et al. Genotypic and Phenotypic Characterization of Erythromycin-Resistant Staphylococcus aureus Isolated from Bovine Mastitis and Humans in Close Contact. Trop Med Infect Dis. 2022;8:26.
25. World Health Organization. Critically Important Antimicrobials for Human Medicine, 6th Revision. https://iris.who.int/bitstream/handle/10665/312266/9789241515528-eng.pdf?sequence=1. 2019;:1–52.
26. Ma GC, Worthing KA, Gottlieb T, Ward MP, Norris JM. Molecular characterization of community-associated methicillin-resistant *Staphylococcus aureus* from pet dogs. Zoonoses Public Health. 2020;67:222–30.
27. Loncaric I, Tichy A, Handler S, Szostak M, Tickert M, Diab-Elschahawi M, et al. Prevalence of Methicillin-Resistant Staphylococcus sp (MRS) in Different Companion Animals and Determination of Risk Factors for Colonization with MRS. Antibiotics. 2019;8:36.
28. Rana EA, Islam MZ, Das T, Dutta A, Ahad A, Biswas PK, et al. Prevalence of coagulase-positive methicillin-resistant *Staphylococcus aureus* and *Staphylococcus pseudintermedius* in dogs in Bangladesh. Vet Med Sci. 2022;8:498–508.
29. Feuer L, Frenzer SK, Merle R, Bäumer W, Lübke-Becker A, Klein B, et al. Comparative Analysis of Methicillin-Resistant Staphylococcus pseudintermedius Prevalence and Resistance Patterns in Canine and Feline Clinical Samples: Insights from a Three-Year Study in Germany. Antibiotics. 2024;13:660.
30. Katakweba AAS, Iversen CM, Tsaxra JB, Muhairwa AP, Moodley A, Olsen JE. Brief communication: Carrier rate, antimicrobial resistance and molecular typing of *Staphylococcus aureus* and *Staphylococcus pseudintermedius* in healthy dogs from Morogoro. Tanzania Vet Dermatol. 2024. https://doi.org/10.1111/vde.13272.
31. Phophi L, Abouelkhair M, Jones R, Henton M, Qekwana DN, Kania SA. The molecular epidemiology and antimicrobial resistance of Staphylococcus pseudintermedius canine clinical isolates submitted to a veterinary diagnostic laboratory in South Africa. PLoS ONE. 2023;18:e0290645.
32. Naziri Z, Majlesi M. Comparison of the prevalence, antibiotic resistance patterns, and biofilm formation ability of methicillin-resistant Staphylococcus pseudintermedius in healthy dogs and dogs with skin infections. Vet Res Commun. 2023;47:713–21.
33. Menandro ML, Dotto G, Mondin A, Martini M, Ceglie L, Pasotto D. Prevalence and characterization of methicillin-resistant Staphylococcus pseudintermedius from symptomatic companion animals in Northern Italy: Clonal diversity and novel sequence types. Comp Immunol Microbiol Infect Dis. 2019;66:101331.
34. Moses IB, Esimone CO, Iroha IR, Rubin JE, Sniatynsky MK, Ribeiro ÁC da S, et al. Antibiotypes and high frequency of toxin genes in methicillin-resistant Staphylococcus pseudintermedius from nares of dogs and dog guardians in Nigeria. Comp Immunol Microbiol Infect Dis. 2022;89:101870.
35. Miszczak M, Korzeniowska-Kowal A, Wzorek A, Gamian A, Rypuła K, Bierowiec K. Colonization of methicillin-resistant Staphylococcus species in healthy and sick pets: prevalence and risk factors. BMC Vet Res. 2023;19:85.
36. Araújo D, Oliveira R, Silva BL, Castro J, Ramos C, Matos F, et al. Antimicrobial resistance patterns of Staphylococcus spp isolated from clinical specimens of companion animals in Northern Portugal, 2021–2023. The Veterinary Journal. 2024;305:106153.
37. Prior CD, Moodley A, Karama M, Malahlela MN, Leisewitz A. Prevalence of methicillin resistance in Staphylococcus pseudintermedius isolates from dogs with skin and ear infections in South Africa. J S Afr Vet Assoc. 2022;93:40a–40h.
38. Dazio V, Nigg A, Schmidt JS, Brilhante M, Mauri N, Kuster SP, et al. Acquisition and carriage of multidrug-resistant organisms in dogs and cats presented to small animal practices and clinics in Switzerland. J Vet Intern Med. 2021;35:970–9.
39. Adiguzel MC, Schaefer K, Rodriguez T, Ortiz J, Sahin O. Prevalence, Mechanism, Genetic Diversity, and Cross-Resistance Patterns of Methicillin-Resistant Staphylococcus Isolated from Companion Animal Clinical Samples Submitted to a Veterinary Diagnostic Laboratory in the Midwestern United States. Antibiotics. 2022;11:609.
40. Papić B, Golob M, Zdovc I, Kušar D, Avberšek J. Genomic insights into the emergence and spread of methicillin-resistant Staphylococcus pseudintermedius in veterinary clinics. Vet Microbiol. 2021;258:109119.
41. Ramos S, Silva V, Dapkevicius M, Igrejas G, Poeta P. Enterococci, from Harmless Bacteria to a Pathogen. Microorganisms. 2020;8:1118.
42. Torres C, Alonso CA, Ruiz-Ripa L, León-Sampedro R, Del Campo R, Coque TM. Antimicrobial Resistance in Enterococcus spp. of animal origin. Microbiol Spectr. 2018;6:10.1128/microbiolspec.arba-0032-2018.
43. Sacramento AG, D. Andrade AC, Teotonio BN, de Oliveira Santos LM, da Silva LCBA, Lincopan N, et al. WHO critical priority van-type vancomycin-resistant Enterococcus in dogs and cats. Prev Vet Med. 2022;202:105614.
44. Raza T, Ullah SR, Mehmood K, Andleeb S. Vancomycin resistant Enterococci: A brief review. J Pak Med Assoc. 2018;68:768–72.
45. Chung YS, Kwon KH, Shin S, Kim JH, Park YH, Yoon JW. Characterization of Veterinary Hospital-Associated Isolates of Enterococcus Species in Korea. J Microbiol Biotechnol. 2014;24:386–93.
46. Ghosh A, Dowd SE, Zurek L. Dogs Leaving the ICU Carry a Very Large Multi-Drug Resistant Enterococcal Population with Capacity for Biofilm Formation and Horizontal Gene Transfer. PLoS ONE. 2011;6:e22451.
47. Darwich L, Seminati C, Burballa A, Nieto A, Durán I, Tarradas N, et al. Antimicrobial susceptibility of bacterial isolates from urinary tract infections in companion animals in Spain. Veterinary Record. 2021;188:e60.
48. Oguttu JW, Qekwana DN, Odoi A. Prevalence and Predictors of Antimicrobial Resistance Among Enterococcus spp. From Dogs Presented at a Veterinary Teaching Hospital, South Africa. Front Vet Sci. 2021;7:589439.
49. Stępień-Pyśniak D, Bertelloni F, Dec M, Cagnoli G, Pietras-Ożga D, Urban-Chmiel R, et al. Characterization and Comparison of Enterococcus spp. Isolates from Feces of Healthy Dogs and Urine of Dogs with UTIs. Animals. 2021;11:2845.
50. Moon B-Y, Ali MdS, Choi J-H, Heo Y-E, Lee Y-H, Kang H-S, et al. Antimicrobial Resistance Profiles of Enterococcus faecium and Enterococcus faecalis Isolated from Healthy Dogs and Cats in South Korea. Microorganisms. 2023;11:2991.
51. Sauer S, Freiwald A, Maier T, Kube M, Reinhardt R, Kostrzewa M, et al. Classification and Identification of Bacteria by Mass Spectrometry and Computational Analysis. PLoS ONE. 2008;3:e2843.
52. Freiwald A, Sauer S. Phylogenetic classification and identification of bacteria by mass spectrometry. Nat Protoc. 2009;4:732–42.
53. Bag S, Saha B, Mehta O, Anbumani D, Kumar N, Dayal M, et al. An Improved Method for High Quality Metagenomics DNA Extraction from Human and Environmental Samples. Sci Rep. 2016;6:26775.
54. Figuerêdo Duarte Moraes M, de Souza Pollo A, Lux Hoppe EG. Filarids (Spirurida: Onchocercidae) in wild carnivores and domestic dogs from the Brazilian Atlantic forest. PLoS Negl Trop Dis. 2022;16:e0010213.
55. Keskimäki M, Eklund M, Pesonen H, Heiskanen T, Siitonen A. EPEC, EAEC and STEC in stool specimens: prevalence and molecular epidemiology of isolates. Diagn Microbiol Infect Dis. 2001;40:151–6.
56. Kumar R, Yadav BR, Singh RS. Genetic Determinants of Antibiotic Resistance in Staphylococcus aureus Isolates from Milk of Mastitic Crossbred Cattle. Curr Microbiol. 2010;60:379–86.
57. Duran N, Ozer B, Duran GG, Onlen Y, Demir C. Antibiotic resistance genes & susceptibility patterns in staphylococci. Indian J Med Res. 2012;135:389–96.

58. Mehrotra M, Wang G, Johnson WM. Multiplex PCR for Detection of Genes for *Staphylococcus aureus* Enterotoxins, Exfoliative Toxins, Toxic Shock Syndrome Toxin 1, and Methicillin Resistance. J Clin Microbiol. 2000;38:1032–5.
59. Essack SY, Hall LMC, Pillay DG, McFadyen ML, Livermore DM. Complexity and Diversity of *Klebsiella pneumoniae* Strains with Extended-Spectrum β-Lactamases Isolated in 1994 and 1996 at a Teaching Hospital in Durban. South Africa Antimicrob Agents Chemother. 2001;45:88–95.
60. Spanu T, Luzzaro F, Perilli M, Amicosante G, Toniolo A, Fadda G, et al. Occurrence of Extended-Spectrum β-Lactamases in Members of the Family *Enterobacteriaceae* in Italy: Implications for Resistance to β-Lactams and Other Antimicrobial Drugs. Antimicrob Agents Chemother. 2002;46:196–202.
61. Dallenne C, Da Costa A, Decré D, Favier C, Arlet G. Development of a set of multiplex PCR assays for the detection of genes encoding important β-lactamases in Enterobacteriaceae. J Antimicrob Chemother. 2010;65:490–5.
62. Kojima A, Ishii Y, Ishihara K, Esaki H, Asai T, Oda C, et al. Extended-Spectrum-β-Lactamase-Producing *Escherichia coli* Strains Isolated from Farm Animals from 1999 to 2002: Report from the Japanese Veterinary Antimicrobial Resistance Monitoring Program. Antimicrob Agents Chemother. 2005;49:3533–7.
63. Rebelo AR, Bortolaia V, Kjeldgaard JS, Pedersen SK, Leekitcharoenphon P, Hansen IM, et al. Multiplex PCR for detection of plasmid-mediated colistin resistance determinants, mcr-1, mcr-2, mcr-3, mcr-4 and mcr-5 for surveillance purposes. Eurosurveillance. 2018;23:17–00672.
64. Poirel L, Walsh TR, Cuvillier V, Nordmann P. Multiplex PCR for detection of acquired carbapenemase genes. Diagn Microbiol Infect Dis. 2011;70:119–23.
65. China B, Pirson V, Mainil J. Typing of bovine attaching and effacing Escherichia coli by multiplex in vitro amplification of virulence-associated genes. Appl Environ Microbiol. 1996;62:3462–5.
66. Ribot EM, Fair MA, Gautom R, Cameron DN, Hunter SB, Swaminathan B, et al. Standardization of Pulsed-Field Gel Electrophoresis Protocols for the Subtyping of Escherichia coli O157:H7, Salmonella, and Shigella for PulseNet. Foodborne Pathog Dis. 2006;3:59–67.
67. Hunter SB, Vauterin P, Lambert-Fair MA, Van Duyne MS, Kubota K, Graves L, et al. Establishment of a Universal Size Standard Strain for Use with the PulseNet Standardized Pulsed-Field Gel Electrophoresis Protocols: Converting the National Databases to the New Size Standard. J Clin Microbiol. 2005;43:1045–50.

Publisher's Note