Topics in Companion An Med 49 (2022) 100638

Research Article

# Multidrug-Resistant Bacteria Isolated From Surgical Site of Dogs, Surgeon's Hands and Operating Room in a Veterinary Teaching Hospital in Brazil

Mareliza P. Menezes[a], Mariana M. Borzi[b], Mayara A. Ruaro[a], Marita V. Cardozo[c,*], Rodrigo C. Rabelo[d], Newton V. Verbisck[e], Paola C. Moraes[a]

*Keywords:*
antimicrobial resistance
community pathogen
enterobacteria
Enterococcus spp
methicillin-resistant Staphylococcus
surgical environment

[a]*Departament of Veterinary Clinic and Surgery, School of Agrarian and Veterinary Sciences, São Paulo State University (UNESP/FCAV), Jaboticabal, SP, Brazil*

[b]*Department of Veterinary Pathology, Faculty of Agricultural and Veterinary Sciences, São Paulo State University, Jaboticabal, São Paulo, Brazil.*

[c]*Minas Gerais State University, Passos, MG, Brazil*

[d]*Intensivet Veterinary Consulting, Brasília, DF, Brazil.*

[e]*Embrapa Beef Cattle, Campo Grande, MS, Brazil*

A B S T R A C T

Surgical environment can play as a source of multidrug-resistance organism, what can pose as a big threat to the patients and health care professionals. This study aimed to evaluate the prevalence and antimicrobial resistance profile of Gram-positive cocci (GPC) and Gram-negative bacilli (GNB) isolated from the surgical environment. All samples were collected during the intraoperative period of clean/clean-contaminated (G1) and contaminated (G2) surgery. A total of 150 samples were collected from the superficial surgical site in the beginning ($n = 30$) and the end ($n = 30$) of the procedure, surgeon's hands before ($n = 30$) and after ($n = 30$) antisepsis, and the surgical environment ($n = 30$). MALDI-TOF MS and antimicrobial susceptibility testing by disk diffusion method were performed for species identification, and determination of the resistance profile. Sixty-eight isolates of GPC and 15 of GNB were obtained. *Staphylococcus* spp. were the most frequent species isolated from surgical site (55.26% [21/38]), surgeon's hands (46.15% [6/13]), and environment (56.67% [17/30]). GPC were mostly resistance to penicillin (85.71% [54/63]), and erythromycin (77.78% [49/63]), and GNB were mostly resistance to cefazolin (58.33% [7/12]), and azithromycin (58.33% [7/12]). High incidence of multidrug resistance was observed in coagulase-negative staphylococci (86.21% [25/29]), coagulase-positive staphylococci (86.67% [13/15]), *Enterococcus* spp. (68.42% [13/19]) and Gram-negative bacilli (60% [9/15]). The high rate of resistance of commensal bacteria found in our study is worrying. Coagulase-negative staphylococci are community pathogens related to nosocomial infections in human and veterinary hospitals, their presence in healthy patients and in veterinary professionals represent an important source of infection in the One Health context. Continuous surveillance and application of antimicrobial stewardship programs are essential in the fight against this threat.

© 2022 Published by Elsevier Inc.

## Introduction

The infection caused by multidrug-resistant bacteria (MDR) presents a main challenge in the One Health context because it increases morbidity, mortality, and the costs related to healthcare.[1-3] The zoonotic potential of most MDR bacterial species related to serious infections highlight veterinary medicine as a relevant axis in the fight against to dissemination of these pathogens.[1,3-9]

Methicillin-resistant *Staphylococcus* (MRS), Vancomycin-Resistant Enterococci, extended-spectrum $\beta$-lactamases (ESBL) Enterobacteriaceae species, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* are a common cause of surgical site infections and hospital-acquired infections in pets and humans. These bacterial groups are the most prevalent microorganism acquired during hospitalization in the intensive care unit in human and veterinary medicine.[3,9-18]

One of the main risk factors to postsurgical infection in dogs is the bacterial colonization of skin, mucous and gastrointestinal tract by MDR bacteria and the presence of these pathogens in the operating room.[13,19,20] Furthermore, the introduction of these strains in the hospital environment poses a big threat to health care professionals, which become carriers and disseminators of MDR bacteria.[17,21,22]

Therefore, this epidemiologic study aimed to evaluate the incidence and antimicrobial resistance profile of Gram-positive cocci (GPC) and Gram-negative bacilli (GNB) present in the superficial surgical site from dogs, surgeon's hands, and the surgical environment during the intraoperative period.

## Material and Methods

### *Case Selection*

Thirty dogs, without antimicrobial therapy in the last 72 hours, undergoing clean/clean-contaminated (G1 [$n = 20$]) and contaminated (G2 [$n = 10$]) surgery[23] in a veterinary teaching hospital of São Paulo state, Brazil, during the 2019 year, were included in this study. Eight surgeons were included in this study. The owners of dogs and surgeons selected for this study signed informed consent for their participation.

### *Patient and Surgical Team Preparation*

The inducing and maintaining anesthesia protocol were performed individually by small animal anesthesiology care according to the *American Society of Anesthesiologists* classification.[24] Cefazolin sodium (25mg/kg [IV]) was administered between 30 and 60 minutes before the surgical incision. Hand's antisepsis was performed with surgical scrub 2% chlorhexidine gluconate or 2% povidone-iodine.

*Corresponding author. Marita V. Cardozo, Minas Gerais State University, Passos, MG, Brazil

*E-mail address:* maritavedovelli@yahoo.com.br (M.V. Cardozo).

*Abbreviations:* C1, Surgeon's hands before antisepsis; C2, Surgeon's hands after antisepsis; CoNS, Coagulase-negative Staphylococci; CoPS, Coagulase-positive Staphylococci; ESBL, Extended-Spectrum $\beta$-lactamases; GPC, Gram-positive cocci; GNB, Gram-negative bacilli; SSS, Superficial surgical site; MDR, Multiple-drug resistant; MRS, methicillin-resistant Staphylococcus

http://dx.doi.org/10.1016/j.tcam.2022.100638
1938-9736/© 2022 Published by Elsevier Inc.

Hair removal by clipping was initiated in the surgical preparation room and finalized after induction in the operating room. Surgical site antisepsis was performed with 2% chlorhexidine solution, 70% isopropyl alcohol, and 0.5% alcoholic chlorhexidine solution.

### *Sample Collection*

Specimens were collected using a dry sterile cotton-tipped swab and transported in a sterile tube with 0.1% peptone salt solution. Sixty samples were collected from the hands surface of the main surgeon immediately before (C1 [$n = 30$]) and after (C2 [$n = 30$]) antisepsis. Swabs were rubbed in a circular motion from the wrists to the fingertips (back and palm), this procedure was repeated 3 times toward each finger by the same person using sterile gloves in all collections.

Immediately after the skin incision ($n = 30$), and previously to cutaneous closure ($n = 30$), the swab was rubbed in the superficial surgical site (SSS) from the patient. The environmental samples were obtained by a Petri plate with Brain Heart Infusion agar (BHI; Oxoid, United Kingdom) that was positioned opened in a support nearly to the surgery table during the procedure. The support has the same height of the surgery table.

### *Bacterial Culture*

Immediately after the procedure, each sample was spread onto MacConkey agar (Oxoid, United Kingdom) and 5% bovine blood agar (Oxoid, United Kingdom) and, together with the BHI environment plate, were incubated at 37°C for 24 hours, under aerobic conditions. Three morphologically distinct colonies were collected from each plate. Each strain was classified by cellular morphology, Gram staining, and presence or absence of spores.[25]

### *Bacterial Identification*

The selected colonies were identified by Matrix-Assisted Laser Desorption Ionization Time-Of-Flight Mass Spectrometry (MALDI-TOF MS) identification. Sample preparation, data acquisition, and analysis were done as previously described by Sauer et al 2008, and Freiwald & Sauer 2009.[26,27] Mass spectra were acquired using an Autoflex III Smartbeam mass spectrometer (Bruker Daltonics) and identified with the manufacturer's software MALDI Biotyper 3.1. Standard Bruker interpretative criteria were applied; scores $\geq$ 2.0 were accepted for species assignment and scores $\geq$ 1.7 but $\leq$ 2.0 for genus identification.

Coagulase test was performed with lyophilized rabbit plasma (Coagu-plasma, Laborclin, Brazil) for the *Staphylococcus* spp. strains not identified for species in MALDI-TOF MS.

### *Antimicrobial Susceptibility Testing*

Isolates were tested by the standard disk diffusion method of Kirby–Bauer[28] on Mueller–Hinton Agar (Oxoid, United Kingdom) incubated at 37°C for 18-24 hours, under aerobic conditions. Inhibition zone diameters were measured following CLSI standard guideline (2018).[29] For enrofloxacin inhibition zone diameters were used CLSI standard guideline (2020).[30] Antimicrobials used in both human and animal medicine were included in susceptibility testing (Table 1). Clindamycin and erythromycin antimicrobial disks were placed at a distance of 15mm to perform inducible clindamycin resistance testing (D-test) in staphylococci strains.

The resistance ratio was obtained by analysing the specific resistance of each identified pathogen to every antimicrobial tested (% R). The intermediate results were considered to be resistant to the data analyses since the outcome of treatment for these cases directly depended on the antimicrobial dose and the site of infection.[29]

**Table 1**
Antimicrobials Used in the Susceptibility Testing

| Bacterial species | Antimicrobial agent |
|---|---|
| *Staphylococcus* spp. | *β-lactams:* Penicillin (10 units), Oxacillin (1 $\mu$g-for CoPS*), Cefoxitin (30 $\mu$g-for CoNS† and CoPS); *Aminoglycosides:* Gentamicin (10 $\mu$g); *Macrolides:* Azithromycin (15 $\mu$g), Erythromycin (15 $\mu$g); *Tetracycline:* Tetracycline (30 $\mu$g); *Fluoroquinolones:* Enrofloxacin (5 $\mu$g); Ciprofloxacin (5 $\mu$g); Norfloxacin (10 $\mu$g); Levofloxacin (5 $\mu$g); *Lincosamides:* Clindamycin (2 $\mu$g); *Sulphonamide:* Trimethoprim-sulfamethoxazole (1.25/23.75 $\mu$g); *Amphenicol:* Chloramphenicol (30 $\mu$g) |
| *Enterococcus* spp. | *β-lactams:* Penicillin (10 units); *Glycopeptide:* Teicoplanin (30 $\mu$g); *Macrolide:* Erythromycin (15 $\mu$g); *Tetracycline:* Tetracycline (30 $\mu$g); *Fluoroquinolones:* Ciprofloxacin (5 $\mu$g), Norfloxacin (10 $\mu$g), Levofloxacin (5 $\mu$g); *Amphenicol:* Chloramphenicol (30 $\mu$g) |
| Enterobacteriaceae | *β-lactams*: Cefazolin (30 $\mu$g), Cefoxitin (30 $\mu$g), Ceftriaxone (30 $\mu$g), Cefotaxime (30 $\mu$g), Ceftazidime (30 $\mu$g), Imipenem (10 $\mu$g); *Aminoglycosides:* Gentamicin (10 $\mu$g); Amikacin (30 $\mu$g); *Macrolide*: Azithromycin (15 $\mu$g); *Tetracycline:* Tetracycline (30 $\mu$g); *Fluoroquinolones:* Enrofloxacin (5 $\mu$g), Ciprofloxacin (5 $\mu$g); Norfloxacin (10 $\mu$g); Levofloxacin (5 $\mu$g); *Sulphonamide:* Trimethoprim-sulfamethoxazole (1.25/23.75 $\mu$g); *Amphenicol:* Chloramphenicol (30 $\mu$g) |
| *Pseudomonas* spp. | *β-lactams:* Ceftazidime (30 $\mu$g), Imipenem (10 $\mu$g); *Aminoglycosides:* Gentamicin (10 $\mu$g), Amikacin (30 $\mu$g); *Fluoroquinolones:* Enrofloxacin (5 $\mu$g), Ciprofloxacin (5 $\mu$g); Norfloxacin (10 $\mu$g); Levofloxacin (5 $\mu$g) |
| *Acinetobacter* spp. | *β-lactams:* Ceftriaxone (30 $\mu$g), Cefotaxime (30 $\mu$g), Ceftazidime (30 $\mu$g), Imipenem (10 $\mu$g); *Aminoglycosides:* Gentamicin (10 $\mu$g), Amikacin (30 $\mu$g); *Tetracycline:* Tetracycline (30 $\mu$g); *Fluoroquinolones:* Ciprofloxacin (5 $\mu$g); Levofloxacin (5 $\mu$g); *Sulphonamide:* Trimethoprim-sulfamethoxazole (1.25/23.75 $\mu$g) |

* Coagulase-positive staphylococci.
† Coagulase-negative staphylococci.

The phenotypic presence of the *mecA* gene (MRS) was assigned to the CoPS which exhibited oxacillin and/or cefoxitin resistance, and to the CoNS which exhibited cefoxitin resistance.[29,31-33] The phenotypic presence of the *ermA* gene ($MLS_B$ phenotype) was assigned to *Staphylococcus* spp. strains that exhibited erythromycin resistance and a D-shape zone around the clindamycin in the area between the 2 disks.[34] Multiple-drug resistance was confirmed when a strain was resistant to at least 1 drug belonging to 3 or more antimicrobial classes.[35,36] The antimicrobial classes used in the Antimicrobial Susceptibility Testing are described in Table 1.

## Results

### *Bacterial Contamination*

GPC and GNB in G1 were observed in 16/20 (80%) and 4/20 (20%) procedures, respectively. In the G2, 8/10 (80%) procedures presented GPC, and 5/10 (50%) procedures presented GNB.

Eighty-three isolates of GPC and GNB were obtained from 150 samples. *Staphylococcus* spp. (53.01% [44/83]) were the most frequently species isolated from the SSS (55.26% [21/38]), surgeon's hands (46.15% [6/13]), and environment (56.67% [17/30]). *Enterococcus* spp. was the second more prevalent species in all 3 sources (22.89% [19/83]).

GNB were isolated from the SSS (40% [6/15]), surgeon's hands (26.67% [4/15]), and environment (33.33% [5/15]). We highlight to the *Proteus mirabilis* isolated from the surgeon's hands after antisepsis, and *P. aeruginosa* and *Acinetobacter schindleri* isolated from the surgeon's hands before antisepsis.

Of which the 15 isolates from de surgeon's hands, 13 were obtained before antisepsis, and 2 were obtained after antisepsis.

All the species isolated from each source in each group are described in Table 2.

**Table 2**
Number of Bacterial Species Obtained From Surgical Environment, Surgeon's Hands and Superficial Surgical Site

| | Superficial surgical site | | Surgeon's hands (before antisepsis) | | Surgeon's hands (after antisepsis) | | Environment | | Total |
|---|---|---|---|---|---|---|---|---|---|
| | G1 | G2 | G1 | G2 | G1 | G2 | G1 | G2 | |
| CoNS* | - | 8 | - | - | - | - | 2 | 4 | 14 |
| CoPS† | 1 | 9 | 1 | - | - | - | 1 | - | 12 |
| *S xylosus* | - | - | - | - | - | - | 2 | - | 2 |
| *S haemolyticus* | - | 1 | - | 1 | - | - | 4 | - | 6 |
| *S hominis* | - | - | - | - | - | - | 2 | - | 2 |
| *S epidermidis* | - | - | 1 | - | - | - | 2 | - | 3 |
| *S capitis* | - | - | - | 1 | - | - | - | - | 1 |
| *S pseudintermedius* | - | 2 | - | - | - | - | - | - | 2 |
| *S aureus* | - | - | 1 | - | - | - | - | - | 1 |
| *S warneri* | - | - | 1 | - | - | - | - | - | 1 |
| *E faecium* | 7 | 2 | 1 | 1 | - | 1 | 4 | 1 | 17 |
| *E faecalis* | - | 2 | - | - | - | - | - | - | 2 |
| *Micrococcus luteus* | - | - | 1 | - | - | - | 2 | 1 | 4 |
| *Aerococcus viridans* | - | - | 1 | - | - | - | - | - | 1 |
| *E coli* | - | 3 | - | - | - | - | - | 1 | 4 |
| *E hermannii* | - | - | - | - | - | - | 1 | - | 1 |
| *Proteus mirabilis* | - | 3 | - | - | - | 1 | - | - | 4 |
| *Pantoea* spp. | - | - | - | 1 | - | - | - | 1 | 2 |
| *Enterobacter cloacae* | - | - | - | - | - | - | 1 | - | 1 |
| *Pseudomonas* spp. | - | - | - | 1 | - | - | - | - | 1 |
| *P stutzeri* | - | - | - | - | - | - | 1 | - | 1 |
| *Acinetobacter schindleri* | - | - | 1 | - | - | - | - | - | 1 |
| Total | 8 | 30 | 8 | 5 | - | 2 | 22 | 8 | 83 |

* CoNS: Coagulase-negative staphylococci.
† CoPS: Coagulase-positive staphylococci.

*Antimicrobial Resistance*

Multiple-drug resistance was observed in 60/78 (76.92%) of all isolates.

CoNS strains presented a higher resistance proportion for penicillin (29/29 [100%]), cefoxitin (22/29 [75.86%]) and erythromycin (22/29 [75.86%]), and CoPS strains for penicillin (12/15 [80%]) and trimethoprim-sulfamethoxazole (12/15 [80%]). *Staphylococcus* spp. strains presented 30/44 (68.18%), 33/44 (75%), and 25/44 (56.82%) of cefoxitin-, erythromycin-, and clindamycin-resistance, respectively. Of the 33 erythromycin resistant *Staphylococcus* spp. strains, 3 (9.09%) were positive to D-test. Thirty-eight of 44 (86.37%) *Staphylococcus* spp. strains were classified as MDR, of which 13/15 (86.67%) were CoPS, and 25/29 (86.21%) were CoNS.

*Enterococcus* spp. presented a high prevalence of erythromycin- and norfloxacin-resistance, both 84.21% (16/19).

GNB presented higher resistance proportion for cefazolin (9/12 [75%]), cefotaxime (7/12 [58.33%]) and azithromycin (7/12 [58.33%]). Multiple-drug resistance was observed in 9/15 (60%) of the GNB strains.

All the resistance ratio are described in Fig. 1.

## Discussion

Our study describes the resistance profile of GPC and GNB isolated from the superficial surgical site, surgeon's hands, and operating room at a veterinary teaching hospital. *Staphylococcus* spp. (44/83 [53.01%]), *Enterococcus* spp. (19/83 [22.89%]), *E. coli* (4/83 [4.82%]) and *P. mirabilis* (4/83 [4.82%]) was the most prevalent pathogen in our samples (Fig. 1). The bacterial species observed in this study were the most prevalent related to the surgical site infections in dogs in Brazil and other countries.[1,13,37-39]

Gobbo et al (2017) and Menezes et al (2020) reported the same pathogens and high proportion of antimicrobial resistance in samples of surgical environment and clinical infections in the same veterinary teaching hospital in São Paulo, Brazil.[40,41] *Staphylococcus* spp. was the most frequent species reported in other veterinary teaching hospitals in other states in Brazil.[37,38] Although the pathogens were similar in these studies, the difference in the bacterial prevalence, mainly between different states, underscores the local and temporal dynamics of bacterial epidemiology.

We observed more CoNS strains (29/44 [65.91%]) than CoPS (15/44 [34.09%]), and CoNS (14/18 [77.78%]) was most prevalent than CoPS (4/18 [22.22%] in G1. This fact may be justified due CoNS strains, mainly *S. epidermidis* and *S. haemolyticus*, are opportunistic pathogens frequently found in dog's and human's skin and usually is not related to infectious in health patient.[9,32,42] These 2 species were the most identified in CoNS group in our study (9/29 [31.03%]). *S. pseudintermedius* and *S. aureus*, CoPS species, are frequently reported in cutaneous infections in dogs, which is usually observed in the superficial surgical site in contaminated surgery, as we see in our isolates in G2.[4,43-45]

MRS strains present resistance to all $\beta$-lactams drugs due to the presence of the *mecA* gene.[31-33] In the present study, cefoxitin-resistance was most prevalent in CoNS (22/29 [75.86%]) than CoPS (8/15 [53.33%]), which is concerning because these species have become potential pathogens related to sepsis in intensive care units (ICU). Furthermore, these strains are associated with biofilm formation and can cause postsurgical infections until 1 year after the procedure.[33,46] Becker et al (2014) reported surveillance of 89% in methicillin-resistance CoNS at humans' hospitals.[45] The high proportion of methicillin-resistance in our strains could lead to surgical antimicrobial prophylaxis failure, which is usually performed with first and second-generation cephalosporins.[45,47]

Three of 33 (9.09%) *Staphylococcus* spp. strains, that were resistant to erythromycin, presented inducible clindamycin resistance (D-test). This fact can occur due to the presence of *erm* gene, which encodes resistance to macrolides, lincosamides, and streptogramin B, called the $MLS_B$ phenotype.[48] The proportion of strains positive to D-test in our study was more than other reports in North America[49,50] and less than health workers in North Africa.[51]

The gastrointestinal tract of animals and humans is colonized by bacteria from the Enterobacteriaceae family and enterococcal species. The presence of these pathogens in the hospital environment and skin may indicate a failure in cleaning and disinfection of these surfaces.[1,52-54]

*Enterococcus faecalis* and *Enterococcus faecium* are the most frequent species of the enterococcal genus. These 2 species were the

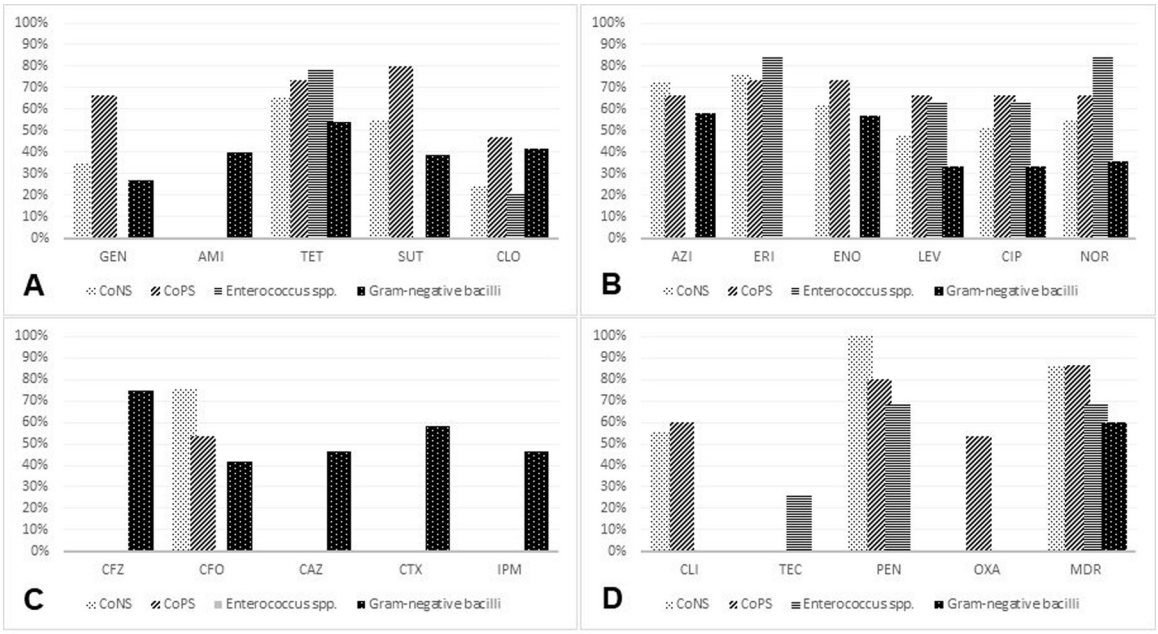



**Fig. 1.** Percentage distribution of antimicrobial resistance in Gram-positive cocci and Gram-negative bacilli obtained from the surgical environment, surgeon's hands, and superficial surgical site during the perioperative period. Amynoglycosides, tetracycline, sulphonamide and Amphenicol (A); Macrolides, lincosamides and fluoroquinolones (B). Cephalosporins and carbapenems (C). Lincosamide, Glycopeptide and Penicillins (D). Legend: GEN = gentamicin, AMI = amikacin, TET = tetracyclin, SUT = trimethoprim-sulfamethoxazole, CLO = Chloramphenicol, AZI = azithromycin, ERI = erythromycin, ENO = enrofloxacin, LEV = levofloxacin, NOR = norfloxacin, CFZ = cefazolin, CFO = cefoxitin, CAZ = ceftazidime, CTX = cefotaxime, IPM = imipenem, CLI = clindamycin, TEC = teicoplanin, PEN = penicillin, OXA = oxacillin, MDR = multidrug-resistance.

most prevalent in our study. They are opportunistic pathogens that could cause nosocomial infections and they could be used as indicators of environmental faecal contamination.[54,55] Our enterococci strains presented a high level of Multiple-drug resistance (13/19 [68.42%]), and teicoplanin-resistance was observed in 5/19 (26.32%) isolates. This drug is the more recently introduced antibiotic from the glycopeptides class used to treat severe human infections.[54,56]

In this study, the most frequent species from the Enterobacteriaceae family were *P. mirabilis* (4/12 [33.33%]) and *E. coli* (4/12 [33.33%]). The presence of these strains in the surgical site and surgeon's hands is probably due to cross-infection and could suggest a failure in antisepsis techniques.[53]

Enrofloxacin-resistance in GNB was the most frequent in the fluoroquinolone group (8/14 [57.14%]). This drug is usually used to treat serious infections caused by enterobacteriaceae strains and *Pseudomonas* spp. in veterinary medicine.[57-59] In our study, the 2 strains of *Pseudomonas* spp. were resistant to enrofloxacin. The empiric administration of enrofloxacin in veterinary routine could contribute to the resistance to other antimicrobials of the same class used to treat human infections, as ciprofloxacin.[57,60]

ESBL production is the main mechanism of resistance in the Enterobacteriaceae family, which results in penicillin, cephalosporin, and aztreonam hydrolysis. $\beta$ -lactamase inhibitors, such as clavulanic acid, sulbactam, and tazobactam could inactivate your action, and these strains are usually sensitive to carbapenem class. The cefotaxime-resistance is the first criteria used to detect the phenotypic production of this enzyme.[29,61] GNB obtained in our study presented a high prevalence (58.33%) of cefotaxime-resistance.

Carbapenemase production is another important resistance mechanism of Enterobacteriaceae. These strains presented resistance to all drugs of the $\beta$-lactam class.[62] The high prevalence of imipenem-resistance (46.67%), a carbapenem class-drug, in our study is worrying because this drug is used to treat serious infections in human medicine. Furthermore, the proportion of MDR strains were high in our strains of CoNS (86.21%), CoPS (86.67%), *Enterococcus* spp. (68.42%) and Gram-negative bacilli (60%), which could mean a high probability of treatment failure.[7]

The presence of MDR strains in healthy patients and the environment is frequently reported in the literature,[63-66] as well as the possibility of transmission to the veterinarians.[21,22] Thus, the patient, health professional, and environment's colonization for these strains, highlighting MRS, Vacomicin-resistance enterococci, ESBL Enterobacteriaceae, and carbapenemase-producing Enterobacteriaceae, could represent a high risk to human and veterinary patient due to therapy failure, and to public health, because they are important silent sources of infection.[1,4,5,46] Furthermore, these strains need, urgently, development for new drugs to treat efficiently these infections.[3]

This study has some limitations. The number of samples was small. The resistance profile was classified just phenotypically, not genotypically. Future studies with other molecular tests, as polymerase chain reaction, 16S rRNA gene sequencing, and pulsed-field gel electrophoresis, are needed to clarified better the species, presence of resistance genes and clonally diversity among isolates.[67-69] The results obtained in the bacteriological culture, MALDI-TO MS, and antimicrobial susceptibility tests are important in clinical care and they are essential in an epidemiological database for the development of antimicrobial stewardship programs and epidemiological surveillance in the One Health context.

## Conclusion

This study identified a high rate of methicillin-resistant CoNS (86.21%), CoPS (86.67%), enterococci resistant to erythromycin (84.21%), GNB resistance to cefotaxime (58.33%), and MDR strains (76.92%). Furthermore, we observed a high resistance proportion in drugs used to treat serious infections in humans, as teicoplanin (26.32%) and imipenem (46.67%). These high rates of resistance are worrying because these strains were collected on skin surfaces without infection, and the pathogens described here are frequently reported surgical site infections and intensive care units in both human and veterinary medicine. Beyond the high potential for therapy failure due to infections for MDR bacteria, we observed the veterinary surgical environment as a potential reservoir and disseminator of these pathogens, mainly of community species, as MRS.

Finally, this study highlights the need for control and prevention in MDR strains transmission in healthcare, which addresses the topic according to the One Health concept and included veterinary medicine as an important cornerstone in the fight against this threat.

## Authors' Contributions

Mareliza Possa de Menezes: Conception and design of study; Acquisition of data; Analysis and interpretation of the data; Writing-Original Draft. Mariana Monezi Borzi: Acquisition of data (bacterial culture, and antimicrobial susceptibility tests); Analysis and interpretation of the data. Mayara Adrielli Ruaro: Acquisition of data (Sample collection, and antimicrobial susceptibility tests); Analysis and interpretation of the data. Marita Vedovelli Cardozo: Supervision; Writing- Reviewing and Editing. Rodrigo Cardoso Rabelo: Analysis and interpretation of the data (Results); Writing- Reviewing and Editing. Newton Valério Verbisck: Acquisition of data (bacterial identification by MALDI-TOF MS); Analysis and interpretation of the data. Paola Castro Moraes: Supervision; Writing- Reviewing and Editing.

## Ethics Approval

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 019707/2017) and Research Ethics Committee (CAAE: 07111419.4.0000.9029, and 39163720.5.0000.9029).

## Funding

This research was funding by National Council for Scientific and Technological (CNPq), grant process number 130822/2018-3, and by São Paulo Research Foundation (FAPESP), grant process number 2019/20585-0.

## References

1. Suthar N, Roy S, Call DR, Besser TE, Davis MA. An individual-based model of transmission of resistant bacteria in a veterinary teaching hospital. *Public Libr Sci One* **9:**1–11, 2014
2. Schwarz S, Enne VI, Duijkeren E. 40 years of veterinary papers in JAC – what have we learnt? *J Antimicrob Chemother* **71:**2681–2690, 2016
3. Mcewen SA, Collignon PJ. Antimicrobial resistance: a one health perspective. *Microbiol Spectr* **6:**1–26, 2018
4. Guardabassi L, Loeber ME, Jacobson A. Transmission of multiple antimicrobial-resistant *Staphylococcus intermedius* between dogs affected by deep pyoderma and their owners. *Vet Microbiol* **98:**23–27, 2004
5. Guardabassi L, Schwarz S, Lloyd DH. Pet animals as reservoirs of antimicrobial-resistant bacteria. *J Antimicrob Chemother* **54:**321–332, 2004
6. Brusselaers N, Vogelaers D, Blot S. The rising problem of antimicrobial resistance in the intensive care unit. *Ann Intensive Care* **1:**1–7, 2011
7. Madec JY, Haenni M, Nordmann P, Poirel L. Extended-spectrum $\beta$-lactamase/AmpC- and carbapenemase producing Enterobacteriaceae in animals: a threat for humans? *Clin Microbiol Infect* **23:**826–833, 2017
8. Grönthal T, Osterblad M, Eklund M, et al. Sharing more than friendship – transmission of NDM-5 ST167 and CTX-M-9 ST69 *Escherichia coli* between dogs and humans in a family, Finland, 2015. *EuroSurveillance* **23:**1–10, 2018
9. Shoen HRC, Rose SJ, Ramsey SA, Morais H, Bermudez LE. Analysis of *Staphylococcus* infections in a veterinary teaching hospital from 2012 to 2015. *Comp Immunol Microbiol Infect Dis* **66:**1–6, 2019
10. Boerlin P, Eugster S, Gaschen F, Straub R, Schawalder P. Transmission of opportunistic pathogens in a veterinary teaching hospital. *Vet Microbiol* **82:**347–359, 2001
11. Gandolfi-Decristophoris P, Regula G, Petrini O, Zinsstag J, Schelling E. Prevalence and risk factors for carriage of multi-drug resistant staphylococci in healthy cats and dogs. *J Vet Sci* **14:**449–456, 2012
12. Turk R, Singh A, Weese JS. Prospective surgical site infection surveillance in dogs. *Vet Surg* **44:**2–8, 2014
13. Andrade N, Schmiedt CW, Cornell K, et al. Survey of intraoperative bacterial contamination in dogs undergoing elective orthopedic surgery. *Vet Surg* **45:**214–222, 2016
14. Reyes K, Bardossy AC, Zervos M. Vancomycin-resistant enterococci: epidemiology, infection prevention and control. *Infect Dis Clin North Am* **30:**953–965, 2016
15. Walther B, Tedin K, Lübke-Becker A. Multidrug-resistant opportunistic pathogens challenging veterinary infection control. *Vet Microbiol* **200:**71–78, 2016
16. Chaudhary R, Thapa SK, Rana JC, Shah PS. Surgical site infections and antimicrobial resistance pattern. *J Nepal Health Res Counc* **15:**120–123, 2017
17. Kaspar U, Lutzau A, Schlattmann A, Roesler U, Köck R, Becker K. Zoonotic multidrug-resistant microorganisms among small companion animals in Germany. *PLoS One* **13:**1–15, 2018
18. Raza T, Ullah SR, Mehmood K, Andleeb S. Vacomycin resistant Enterococci: a brief review. *J Pakistan Med Assoc* **68:**768–772, 2018
19. Chauveaux D. Preventing surgical-site infections: measures other than antibiotics. *Orthop Traumatol Surg Res* **101:**S77–S83, 2015
20. Burgess BA. Prevention and surveillance of surgical infections: a review. *Vet Surg* **48:**284–290, 2019
21. Paul NC, Moodley A, Ghibaudo G, Guardabassi L. Carriage of methicillin-resistant *Staphylococcus pseudintermedius* in small animal veterinarians: indirect evidence of zoonotic transmission. *Zoonoses Public Health* **58:**533–539, 2011
22. 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* **24:**434–446, 2017
23. WHO. Global guidelines for the prevention of surgical site infection. *Geneva* **1:**186, 2016
24. Mayhew D, Mendonca V, Murthy BVS. A review of ASA physical status – historical perspectives and modern developments. *Anaesthesia* **74:**373–379, 2019
25. Barrow GI, Feltham RKA. *Cowan and Steel's: Manual for the Identification of Medical Bacteria*. 3rd ed. Cambridge: Cambridge University; 1993
26. Sauer S, Freiwald A, Maier T, et al. Classification and identification of bacteria by mass spectrometry and computational analysis. *PLoS One* **3:**e2843, 2008
27. Freiwald A, Sauer S. Phylogenetic classification and identification of bacteria by mass spectrometry. *Nat Protoc* **4:**732–742, 2009
28. Bauer A, Kirby WMN, Sherris JC, Turk M. Antibiotic susceptibility testing by a standardized single disc method. *Am J Pathol* **45:**493–496, 1996
29. CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests, CLSI Document MO2-A10*. Wayne: Clinical and Laboratory Standard Institute; 2018. p. 240
30. CLSI. *Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals—Fifth Edition: Approved Standard, CLSI Document VET01S-ED5. Wayne*. Clinical and Laboratory Standard Institute; 2020. p. 250
31. Bemis DA, Jones RD, Frank LA, Kania SA. Evaluation of susceptibility test breakpoints used to predict *mecA*-mediated resistance in *Staphylococcus pseudintermedius* isolated from dogs. *J Vet Diagn Invest* **21:**53–58, 2009
32. Wu MT, Burnham CAD, Westblade LF, et al. Evaluation of oxacillin and cefoxitin disk and MIC breakpoints for prediction of methicillin resistance in human and veterinary isolates of *Staphylococcus intermedius* group. *J Clin Microbiol* **54:**535–542, 2016
33. Naccache SN, Callan K, Burnham CD, Wallace MA, Westblade LF, Bard JD. Evaluation of oxacillin and cefoxitin disk diffusion and microbroth dilution methods for detecting *mecA*-mediated $\beta$-lactam resistance in contemporary *Staphylococcus epidermidis* isolates. *J Clin Microbiol* **57:**1–17, 2019
34. Fiebelkorn KR, Crawford SA, McElmeel ML, Jorgensen JH. Practical disk diffusion method for detection of inducible clindamycin resistance in *Staphylococcus aureus* and coagulase-negative staphylococci. *J Clin Microbiol* **41:**4740–4744, 2003
35. Magiorakos AP, Srinivasan A, Carey RB, 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* **18:**268–281, 2012
36. Basak S, Singh P, Rajurkar M. Multidrug resistant and extensively drug resistant bacteria: a study. *J Pathog* **2016:**1–5, 2016
37. Fernando FS, Silva KR, Vignoto VKC, De Conti JB, Pachalu JR, Wosiacki SR. Microbial assessment of site surgical relating to time of procedure and antimicrobial resistance in dogs and cats. *Vet Sci Public Health* **2:**26–33, 2015
38. Murta AR, Abreu Jr NB, Oliveira LS, et al. Epidemiological profile and microbiological analysis of surgical site infection in human and pet patients. *Braz J Vet Res* **35:**652–658, 2015
39. Verwilghen D, Singh A. Fighting surgical site infections in small animals-are we getting anywhere? *Vet Clin* **45:**243–276, 2015
40. Gobbo JL, Cardozo MV, Lacerda LCC, et al. Evaluation of operating room environment contamination and efficacy of 2% chlorhexidine for surgical hand scrubbing before and after gowning and gloving. *Acta Scientiae Veterinariae* **45:**1484, 2017
41. 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* **42**:100489, 2020
42. Kern A, Perreten V. Clinical and molecular features of methicillin-resistant, coagulase-negative staphylococci of pets and horses. *J Antimicrob Chemother* **68:**1256–1266, 2013
43. Howe LM, Boothe HW. Antimicrobial Use in the surgical patient. *Vet Clin* **36:**1049–1060, 2006
44. Hillier A, Lloyd DH, Weese JS, et al. Guidelines for the diagnosis and antimicrobial therapy of canine superficial bacterial folliculitis (Antimicrobial Guidelines Working Group of the International Society for Companion Animal Infectious Diseases). *Vet Dermatol* **25:**163., 2014. e43
45. Singh A, Weese JS (2017) Wound infections and antimicrobial use. In: Johnston SA, Tobias KM (Eds) Veterinary Surgery Small Animal, vol. 1, 2nd edn. Elsevier, New York, pp 530-548.
46. Becker K, Hellman C, Peters G. Coagulase-negative staphylococci. *Clin Microbiol Rev* **27:**870–926, 2014
47. Boothe DM, Boothe Jr HW. Antimicrobial considerations in the perioperative patient. *Vet Clin* **45:**585–608, 2015

48. Fernandes CJ, O'Sullivan MVN, Cai Y, et al. Agar dilution method for detection of inducible clindamycin resistance in *Staphylococcus* spp. *J Clin Microbiol* **45:**4018–4020, 2007
49. Faires MC, Gard S, Aucoin D, Weese JS. Inducible clindamycin resistance in methicillin-resistant *Staphylococcus aureus* and methicillin resistant *Staphylococcus pseudintermedius* isolates from dogs and cats. *Vet Microbiol* **139:**419–420, 2009
50. Gold RM, Lawhon SD. Incidence of inducible clindamycin resistance in *Staphylococcus pseudintermedius* from dogs. *J Clin Microbiol* **51:**4196–4199, 2013
51. Mahmoud AM, Albadawy HS, Bolis SM, Bilal NE, Ahmed AO, Ibrahim ME. Inducible clindamycin resistance and nasal carriage rates of *Staphylococcus aureus* among healthcare workers and community members. *Africa Health Sci* **15:**861–867, 2015
52. Cinquepalm V, Monno R, Fumarola L, et al. Environmental contamination by dog's faeces: a public health problem? *Int J Environ Res Public Health* **10:**72–84, 2013
53. Drzewiecka D. Significance and roles of *Proteus* spp. bacteria in natural environments. *Microb Ecol* **72:**741–758, 2016
54. 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* **6:**1–41, 2018
55. Boehm AB, Sassoubre LM. Enterococci as indicators of environmental fecal contamination. In: Gilmore MS, Clewell DB, Ike Y, Shankar N, editors. *Enterococci: From Commensals to Leading Causes of Drug Resistant Infection*, Boston: Massachusetts Eye and Ear Infirmary; 2014. p. 101–122
56. García-Solache M, Rice LB. The *Enterococcus*: a model of adaptability to its environment. *Clin Microbiol Rev* **32:**e00018–e00058, 2019
57. Papich MG. Antimicrobials, susceptibility testing, and minimum inhibitory concentrations (MIC) in veterinary infection treatment. *Vet Clin* **43:**1079–1089, 2013
58. Feng X, Zhang Z, Li X, et al. Mutations in *gyrB* play an important role in ciprofloxacin-resistant *Pseudomonas aeruginosa*. *Infect Drug Resist* **12:**261–272, 2019
59. Pang Z, Raudonis R, Glick BR, Lin TJ, Cheng Z. Antibiotic resistance in *Pseudomonas aeruginosa*: mechanisms and alternative therapeutic strategies. *Biotechnol Adv* **37:**177–192, 2019
60. Barrasa JLM, Gomez LP, Lama ZG, Junco MTT. Antibacterial susceptibility patterns of *Pseudomonas* strains isolated from chronic canine otitis externa. *J Vet Med B Infect Dis Vet Public Health* **47:**191–196, 2000
61. Laudy AE, Róg P, Smolinska-Krol K, et al. Prevalence of ESBL-producing *Pseudomonas aeruginosa* isolates in Warsaw, Poland, detected by various phenotypic and genotypic methods. *PLoS One* **12:**1–15, 2017
62. Seibert G, Horner R, Meneghetti BH, Righi RA, Dal Forno NLF, Salla A. Infecções hospitalares por enterobactérias produtoras de *Klebsiella pneumoniae* carbapenemase em um hospital escola. *Einstein (São Paulo)* **12:**282–286, 2014
63. Chah KF, Gómez-Sanz E, Nwanta JA, et al. Methicillin-resistant coagulase-negative staphylococci from healthy dogs in Nsukka, Nigeria. *Braz J Microbiol* **45:**215–220, 2014
64. Priyantha R, Gaunt MC, Rubin JE. Antimicrobial susceptibility of *Staphylococcus pseudintermedius* colonizing healthy dogs in Saskatoon, Canada. *Can Vet J* **57:**65–69, 2016
65. Dupouy V, Abdelli M, Moyano G, et al. Prevalence of beta-lactam and quinolone/fluoroquinolone resistance in enterobacteriaceae from dogs in France and Spain—characterization of ESBL/pAmpC isolates, genes, and conjugative plasmids. *Front Vet Sci* **6:**279, 2019
66. Ortega-Paredes D, Haro M, Leoro-Garzón P, et al. Multidrug-resistant *Escherichia coli* isolated from canine faeces in a public park in Quito, Ecuador. *J Global Antimicrob Resist* **18:**263–268, 2019
67. Ribot EM, Fair MA, Gauton R, 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* **3:**59–67, 2006
68. Woo PCY, Lau SKP, Teng JLL, Tse H, Yuen KY. Then and now: use of 16S rRNA gene sequencing for bacterial identification and discovery of novel bacteria in clinical microbiology laboratories. *Clin Microbiol Infect* **14:**908–934, 2008
69. Srinivasan R, Karaoz U, Volegova M, et al. Use of 16S rRNA gene for identification of a broad range of clinically relevant bacterial pathogens. *PLoS One* **10:**1–22, 2015