

Communication

Multidrug-Resistant *Enterococcus faecium* and *Enterococcus faecalis* Isolated from Dogs and Cats in Southern Brazil

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Abstract: *Enterococcus* spp. are isolated from infections of domestic animals and can present resistance to different antimicrobials. This study aimed to identify the main species of *Enterococcus* and determine the phenotypic resistance to antimicrobials of isolates of this bacterial genus obtained from dogs and cats admitted to a veterinary hospital in southern Brazil. A total of 57 *Enterococcus* spp. were isolated from different clinical samples (urine, feces, ears and skin) in domestic animals admitted to the hospital over a period of three years (2016 to 2019). MALDI-TOF results demonstrated the occurrence of *Enterococcus faecium* (39; 68.4%), *Enterococcus faecalis* (17; 29.8%) and *Enterococcus avium* (1; 1.8%). In an overall analysis, resistance was observed to the antimicrobials rifampicin (46; 80.7%), tetracycline and streptomycin (42; 73.7%), ampicillin and imipenem (41; 71.9%), erythromycin (39; 68.4%), gentamicin (38; 66.7%), ciprofloxacin (36; 63.2%), norfloxacin (32; 56.1%), nitrofurantoin (10; 17.5%) and chloramphenicol (9; 15.7%). None of the *Enterococcus* spp. showed resistance to vancomycin and linezolid. Multidrug resistance (MDR) was detected in 45 (78.9%) isolates. In conclusion, *E. faecium* and *E. faecalis* with MDR are frequent in infections of hospitalized dogs and cats from southern Brazil.

Keywords: multidrug resistance (MDR); MALDI-TOF; pets; veterinary hospital



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1. Introduction

Enterococcus spp. are Gram-positive commensal bacteria generally present in the intestine of animals. In veterinary hospitals, this bacterial genus is also frequently detected in cutaneous and systemic infections of small pets, such as dogs and cats [1]. Nosocomial infections caused by this genus can result in septicemia, endocarditis and urinary tract diseases in hospitalized animals [2,3]. Furthermore, pets are reservoirs of *Enterococcus* spp., including strains associated with human infections [4].

Currently, the genus *Enterococcus* has more than 50 bacterial species [5]. *Enterococcus faecalis* and *Enterococcus faecium* have been more frequently isolated from animals in veterinary hospitals [6–8]. Antimicrobial resistance is also a remarkable characteristic of many *Enterococcus* spp. isolates. The ability of this bacterium to acquire antimicrobial resistance and transfer it to other bacterial species makes it difficult to control and treat the enterococcal infections. Previous studies have already demonstrated the high resistance of this genus to a wide range of antimicrobial agents from different classes [2,9,10]. Antimicrobial resistance was even demonstrated for “last choice” antimicrobials, such as vancomycin and linezolid [11,12].

The present study aimed to determine the bacterial species and phenotypic antimicrobial resistance of *Enterococcus* spp. isolated from dogs and cats admitted to a veterinary faculty hospital in southern Brazil.

2. Materials and Methods

A total of 2223 bacterial isolates were obtained from different animals (mainly dogs and cats) with bacterial infections, which were routinely treated at the Veterinary University Hospital of the Lutheran University of Brazil (ULBRA, Canoas, RS, Brazil), Southern Brazil, in the period from January 2016 to June 2019 (Table 1). Clinical samples included urine and swabs of ears, wound secretions and skin. These samples were collected and immediately streaked onto blood agar (Acumedia®, Lansing, MI, USA) or MacConkey plates (Kasvi®, São José dos Pinhais, Brazil) and incubated overnight (18 to 24 h) at 37 °C. Swabs and urine aliquots of 0.5 mL of each sample were kept in Brain Heart Infusion (BHI, Himedia®, Mumbai, India) until the end of laboratory analysis.

Table 1. Number of *Enterococcus* spp. isolates according to the year.

Year	Total Bacterial <i>n</i>	<i>Enterococcus</i> spp. <i>n</i> (%)	Dogs <i>n</i> (%)	Cats <i>n</i> (%)	Horses <i>n</i> (%)	Cows <i>n</i> (%)	Missing <i>n</i> (%)
2016	485	59 (12.2)	48 (81.3)	5 (8.5)	5 (8.5)	1 (1.7)	0 (0)
2017	646	76 (11.8)	59 (77.6)	10 (13.1)	6 (7.9)	0 (0)	1 (1.3)
2018	778	68 (8.7)	53 (77.9)	9 (13.2)	3 (4.4)	0 (0)	3 (4.4)
2019	314	29 (9.2)	20 (68.9)	6 (20.7)	2 (5.8)	1 (3.4)	1 (3.4)
Total	2223	232 (10.4)	180 (77.6)	30 (12.9)	16 (6.9)	2 (0.9)	5 (2.1)

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Bacterial isolates were classified as *Enterococcus* spp. or another genus according to traditional bacteriological analysis. All isolates identified as *Enterococcus* spp. presented the microscopic (spherical or ovoid Gram-positive cells arranged in pairs or chains) and biochemical (negative catalase reaction, growth in broth containing 6.5% NaCl, esculin hydrolysis in the presence of 40% bile salts) characteristics expected for this genus, as previously described [13].

In the study period, 57 randomly isolates of *Enterococcus* spp. were selected and stored at −20 °C in BHI agar supplemented with 30% glycerol. These bacterial species were further identified by Matrix-Assisted Laser Desorption and the Ionization Time-Of-Flight technique (MALDI-TOF). Briefly, each individual colony was transferred onto main-spectrum-profile 96 target polished steel (Bruker Daltonik, Bremen, Germany) using a toothpick. To crystalize bacterial components, 1 µL of saturated α -cyano-4-hydroxycinnamic acid matrix solution in 50% acetonitrile-2.5% trifluoroacetic acid was overlaid on each well and air-dried at room temperature (25 °C). Spectrum profiles of isolates were obtained using a mass spectrometer (Bruker Daltonik) with default identification standard settings. Bacteria were identified by analyzing spectra according previously described [14].

The antimicrobial resistance profile against 13 antimicrobials frequently used in veterinary medicine was further evaluated using the Kirby–Bauer disk diffusion method. The following antimicrobials were included in the analysis: ampicillin (10 µg), ciprofloxacin (5 µg), chloramphenicol (30 µg), erythromycin (15 µg), gentamycin (120 µg), imipenem (4 µg), linezolid (30 µg), nitrofurantoin (300 µg), norfloxacin (10 µg), rifampicin (5 µg), streptomycin (300 µg), tetracycline (30 µg), and vancomycin (30 µg) [15]. Multidrug resistance (MDR) was defined as non-susceptibility to at least three agents from three or more different antimicrobial categories, while not-MDR was defined as susceptibility to all drugs tested or non-susceptibility to a maximum of agents from one to two antimicrobial categories [16].

3. Results

The bacteriological records demonstrated that 232 (10.4%) of the 2223 isolates were identified as *Enterococcus* spp. The percentage per year ranged from 8.7% in 2018 to 12.2% in 2016. *Enterococcus* spp. was isolated from dogs, cats and other animals (Table 1). The 57 previously stored *Enterococcus* spp. isolates (47 from dogs and 10 from cats) were further characterized with MALDI-TOF. *E. faecium* was the predominant species ($n = 39$; 68.4%), followed by *E. faecalis* ($n = 17$; 29.8%) and *E. avium* ($n = 1$; 1.8%) (Table 2).

Table 2. Number and frequency of *Enterococcus* species isolates in dogs and cats.

	<i>E. faecium</i>	<i>E. faecalis</i>	<i>E. avium</i>	Total
Dogs	32 (68.1%)	14 (29.8%)	1 (2.1%)	47
Cats	7 (70%)	3 (30%)	0 (0%)	10
Total	39 (68.4%)	17 (29.8%)	1 (1.8%)	57

The antimicrobial susceptibility profiles of these *Enterococcus* spp. isolates demonstrated that 56 (98.2%) were resistant to at least one drug (Figure 1), i.e., only one sample (*E. faecalis* isolate 107/19) presented an overall susceptible profile to all tested antimicrobials (it also presented an intermediate resistance to ciprofloxacin and chloramphenicol). *E. faecium* isolates presented a high frequency of resistance to streptomycin, ampicillin and imipenem ($n = 38$; 97.4%), followed by rifampicin ($n = 35$; 89.7%), erythromycin and gentamycin ($n = 34$; 87.2%), tetracycline ($n = 33$; 84.6%), ciprofloxacin ($n = 32$; 82.1%), norfloxacin ($n = 28$; 71.8%), nitrofurantoin ($n = 10$; 25.6%) and chloramphenicol ($n = 6$; 15.4%). In addition, there was also intermediate resistance for six antibiotics: chloramphenicol ($n = 10$; 25.6%), norfloxacin ($n = 7$; 23.3%), ciprofloxacin ($n = 6$; 15.4%), erythromycin ($n = 4$; 10.2%), rifampicin ($n = 2$; 5.1%) and nitrofurantoin ($n = 1$; 2.6%). A total of 38 *E. faecium* isolates (97.4%) presented an MDR profile. On the contrary, *E. faecalis* isolates frequently presented resistance to rifampicin ($n = 11$; 64.7%), followed by tetracycline ($n = 8$; 47%), erythromycin ($n = 5$; 29.4%), gentamycin, streptomycin, ciprofloxacin and norfloxacin ($n = 4$; 23.5%), chloramphenicol ($n = 3$; 17.6%), imipenem and ampicillin ($n = 2$; 11.8%). There were also isolate samples with intermediate resistance for ciprofloxacin ($n = 8$; 47%), norfloxacin ($n = 6$; 35.2%), erythromycin ($n = 5$; 29.4%), rifampicin ($n = 3$; 17.6%), streptomycin, chloramphenicol and tetracycline ($n = 1$; 5.9%). Six (35.3%) *E. faecalis* isolates showed resistance to three or more antibiotics (MDR profile). The unique *E. avium* isolate was obtained from a dog sample and presented resistance to ampicillin, imipenem and tetracycline, as well as an MDR profile (Figure 1).

The overall analysis according to the antimicrobial classes demonstrated a high frequency of resistance to rifampicin (80%), β -lactams, aminoglycosides and tetracyclines (70–79%), and fluoroquinolones and macrolides (55–69%). The resistance to phenols, nitrofurans and oxazolidinones was below 20%. Noteworthy, all *Enterococcus* spp. were susceptible to vancomycin and linezolid. In a comparative analysis between the two frequent *Enterococcus* species, *E. faecium* presented a more concerning profile, with resistance to two (only one isolate) to eleven (two isolates) out of the thirteen tested antimicrobials (mean = 6.1; median = 9), while *E. faecalis* presented resistance to one (in only one isolate) to nine (also one isolate) antimicrobials (mean = 1.7; median = 1), but there was also one isolate with resistance to nine drugs (Figure 1). Importantly, *E. faecium* had a higher frequency of MDR (97.4%) than *E. faecalis* (35.3%).

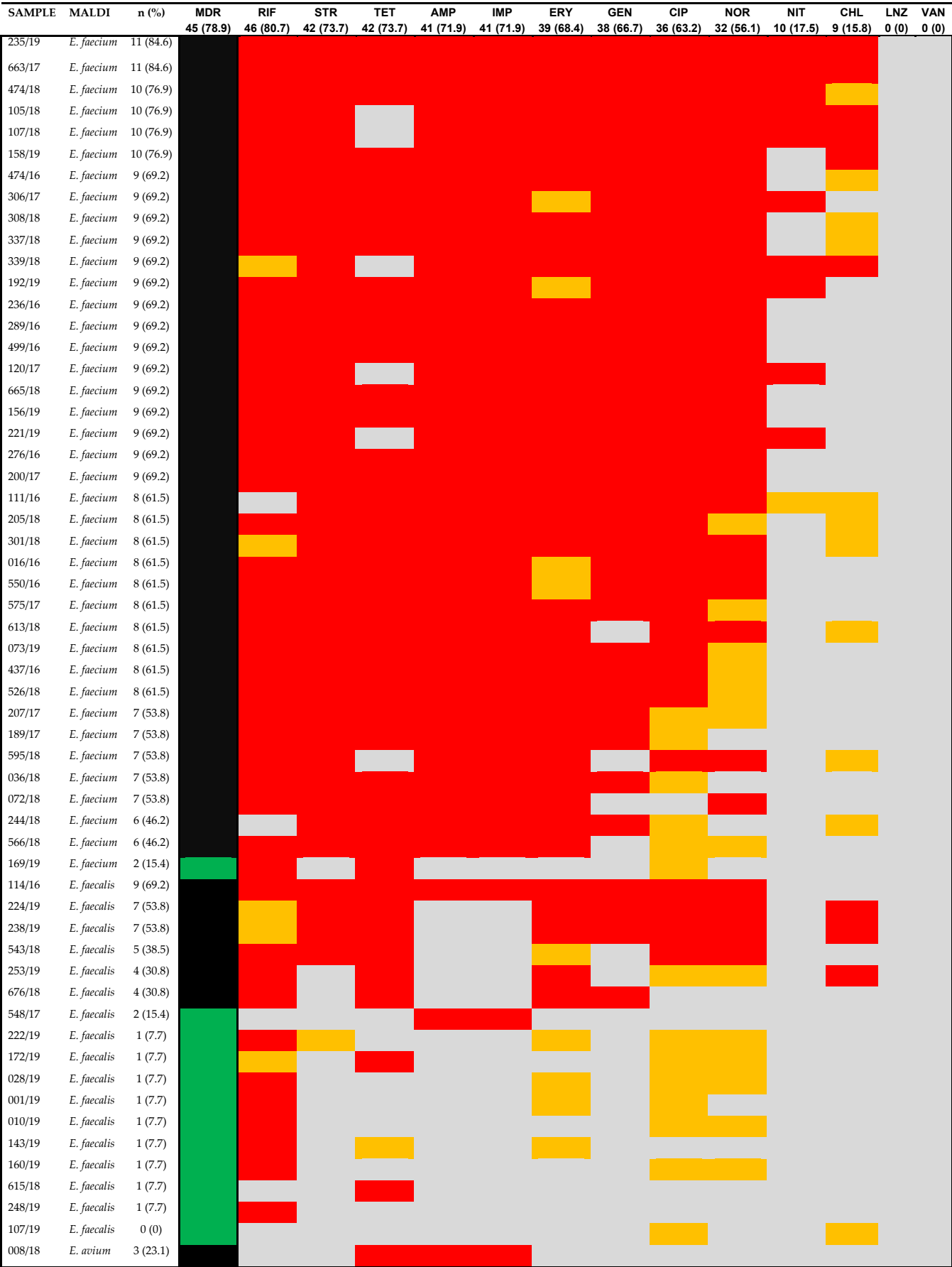


Figure 1. Multiple and individual non-susceptibility profiles of the tested antimicrobials of the *Enterococcus* isolates from hospitalized dogs and cats; *n* (%) is the total number of non-susceptible tested

antimicrobials (%). Black—multidrug-resistant (MDR) isolates. Green—not-MDR isolates. Red—complete resistance. Yellow—intermediate resistance. Gray—no resistance (susceptible). RIF = rifampicin; STR = streptomycin; TET = tetracycline; AMP = ampicillin; IMP = imipenem; ERY = erythromycin; GEN = gentamycin; CIP = ciprofloxacin; NOR = norfloxacin; NIT = nitrofurantoin; CHL = chloramphenicol; LNZ = linezolid; VAN = vancomycin.

4. Discussion

Enterococcus spp. are usually recognized as commensal bacteria. However, different *Enterococcus* species can cause diarrhea, endocarditis and even septicemia in dogs and cats [8]. In addition, cystitis and skin lesions may result from opportunistic *Enterococcus* spp. present in the normal buccal microbiota of domestic animals [17]. This ability of *Enterococcus* spp. to cause infections has been related to the intrinsic robustness of this bacterial genus, persisting in animal populations and hospital environments [18,19]. This concerning Gram-positive bacteria species was frequently detected in hospitalized dogs and cats in this study, reaching a total of 10.4% of the bacterial isolates in the routine microbiological service at a veterinary teaching hospital over a period of three years. *Enterococcus* spp. isolates were obtained from different animal species and distinct tissue systems, such as urinary, otological and cutaneous systems. Previous studies have shown similar frequencies of isolation (between 5 and 25%) of *Enterococcus* sp. in different animal clinical specimens [2,18,20].

In the analysis of *Enterococcus* species, the frequency of *E. faecium* (68.4%) was higher than *E. faecalis* (29.8%), with similar results in both sampled animal species (dogs and cats). This result contrasts to other studies that demonstrated a higher frequency of *E. faecalis* than *E. faecium* in veterinary hospitals as well as in humans [8,18,20–22]. In addition, one isolate of *E. avium* was detected in a dog sample. This bacterial species had already been detected in pets and was also demonstrated to be associated with an emphysematous pyometra in a dog [8,23].

Enterococci also serve as the indicator of bacterial resistance both in human and veterinary resistance surveillance systems [24,25]. Therefore, the present study also focused on the evaluation of the bacterial antimicrobial resistance profiles of these hospital isolates. They demonstrated resistance to different classes: rifampicin, β -lactams, aminoglycosides and tetracyclines. For *E. faecium*, the median resistance was to 9 out of 13 antibiotics tested (including 1 with resistance to 12 antimicrobials), with an MDR profile in 38 out of 39 (97.4%) isolates. A previous study demonstrated that dogs and cats treated with antibiotics are commonly colonized with antimicrobial-resistant enterococci [20]. *E. faecalis* had a much lower resistance level, with a median of one out of thirteen antimicrobials tested and with an MDR profile in six of the seventeen isolates (35.3%). *E. faecium* is less susceptible to antimicrobials than *E. faecalis*, with more than half expressing resistance to ampicillin and aminoglycosides. This high level of antibiotic resistance has already been observed in other animal health care facilities and in human medical settings [20,24].

Enterococci are pathogens of huge clinical concern for the development and dissemination of MDR [25,26]. Other studies seem to reinforce this scenario, demonstrating an MDR profile in more than 60% of the enterococci isolates in the last decade [7,9,24]. *E. faecium* is also considered a bacterial pathogen by the World Health Organization with priority status, as it presents unique resistance profiles and constitutes a central concern in the global health scenario [27]. The results presented here are concerning, since they also demonstrate the high frequency of multidrug-resistant *E. faecium* in pets from Brazil, many of them living in close contact with humans. Noteworthy, all strains here-characterized were susceptible to vancomycin—the antibiotic most widely used to control the MDR of bacteria in hospital clinical settings. Previous reports have even demonstrated the occurrence of vancomycin resistance in enterococci isolated from domestic animals [28,29]. Therefore, ongoing surveillance studies of these bacterial pathogens are needed in both human and veterinary medical settings.

The present study also highlights the importance of MALDI-TOF for the identification of enterococci, as previously reported [14]. The use of this technique can be associated with molecular biology methods, which have also been very useful in the identification of bacterial infections. PCR methods are described for the detection of the genus *Enterococcus* and its main species [30]. More recently, next-generation sequencing approaches have also made it possible to obtain important genomic information on the pathogenic mechanisms, virulence and antimicrobial resistance of this concerning bacterial genus [30]. Taken together, this arsenal of molecular diagnostic methods can help in the rapid diagnosis of any enterococci infection to initiate appropriate therapy as early as possible, reducing the use of unnecessary antibiotics.

5. Conclusions

In conclusion, we demonstrated the occurrence of two main enterococci species (*E. faecium* and *E. faecalis*) in infections of hospitalized dogs and cats. Also, several enterococci isolates presented an MDR profile. The present findings are also important for public health, since the identification and characterization of antimicrobial resistance profiles evidence which drugs have higher and lower resistance, maximizing the success of empirical therapies. Finally, these results reinforce the importance of constantly surveilling the enterococci resistance profiles in veterinary facilities.

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Conflicts of Interest: A.S.K.F. and N.I. work at the company Simbios Biotecnologia. D.K. is a CNPq scholarship holder and develops projects at the same company. The other authors declare that the research was carried out in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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