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Multidrug-resistant *Enterococcus faecalis* isolated from ornamental animals feed †

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Abstract: *Enterococcus faecalis* is one of the species most strongly associated with cases of nosocomial infections. This pathogen is resistant to several antimicrobial classes, having an enormous capacity to acquire and transfer resistance genes. The objective of this work was to evaluate the level of antibiotic resistance *Enterococcus faecalis* isolates recovered from samples of food supplied to ornamental animals. A total of 57 samples of ornamental animal feed (birds, fish, mammals and reptiles) were collected between February and December 2020. Hundred and three *Enterococcus faecalis* putative isolates, obtained from Slanetz-Bartley and Kanamycin azide aesculin agar selective plates, were recovered from 15 birds, 9 from fish and 4 from reptile feed samples. The identification of isolates was confirmed by standard biochemical tests. Antimicrobial susceptibility was performed using 14 antimicrobial agents by the Kirby-Bauer disk diffusion method, according to the Clinical and Laboratory Standards Institute standards. *Enterococcus faecalis* isolates showed a higher prevalence of rifampicin resistance (77.7%). Additionally, these isolates also demonstrated resistance to erythromycin (48.5%) and ciprofloxacin (37.9%). None of the isolates showed resistance to gentamicin and streptomycin. Almost half of the isolates (47.6%) showed multidrug-resistance profile; 23.3% showed resistance to 3 different antimicrobial classes, 6.8% to 4 and 17.5% to 5 or more classes. In conclusion, these results indicated a significant presence of *E. faecalis* in the feeding of ornamental animals, as well as, multidrug-resistant isolates, becoming a public health problem given the proximity and interaction of humans with these animals.

Keywords: *Enterococcus faecalis*; feed; ornamental animals; antibiotic resistance

1. Introduction

Enterococcus faecalis is one of the species most evaluated and studied because it is predominantly found in humans, and is strongly associated with cases of nosocomial infections [1], such as bacteremia, septicemia, urinary tract infections, wound infections, meningitis and endocarditis [2-4]. This pathogen is resistant to several antimicrobial classes, such as β-lactams, aminoglycosides, glycopeptides, lincosamides, streptogramins and also trimethoprim sulfamethoxazole [5], having an enormous capacity to acquire and transfer resistance genes [6]. In order to induce inflammatory processes and increase antimicrobial resistance, *E. faecalis* produces virulence factors and biofilms [7,8].

Antimicrobial resistance is a serious problem with an impact on wildlife, the environment and especially on human health. Since the inappropriate use of antibiotics facilitates the spread of resistance and hinders the treatment of infectious diseases in the human and animal population. Ornamental animals are considered as strong reservoirs of antibiotic-resistant microorganisms and, due to close contact and the increased interaction with humans, they can transfer them to humans [9].

The aim of this work was to evaluate the level of antibiotic resistance *Enterococcus faecalis* isolates recovered from samples of food supplied to ornamental animals.

2. Materials and Methods

2.1. Isolates

A total of 57 samples of ornamental animal feed (birds, fish, mammals and reptiles) were collected between February and December 2020. Hundred and three *Enterococcus faecalis* putative isolates, obtained from Slanetz-Bartley agar **(**Liofilchem® s.r.l., Italy) and Kanamycin aesculin azide agar (Liofilchem® s.r.l., Italy) selective plates, were recovered from 15 birds, 9 from fish and 4 from reptile feed samples. The identification of isolates was confirmed by standard biochemical test, like Gram stain, catalase and 6.5% NaCl tests.

2.2. Antimicrobial susceptibility testing

Antimicrobial susceptibility was performed using 14 antimicrobial agents , such as Ampicilin (10 μg), Vancomycin (30 μg), Teicoplanin (30 μg) , Tetracycline (30 μg), Erythromycin (15 μg), Ciprofloxacin (5 μg), Chloramphenicol (30 μg), Quinupristin/ dalfopristin (15 μg), Nitrofurantoin (300 μg), Rifampicin (5 μg), Fosfomycin (200 μg), Gentamicin (120 μg), Streptomycin (300 μg) and Linezolid (30 μg), by the Kirby-Bauer disk diffusion method, according to the Clinical and Laboratory Standards Institute standards [10].

Each *E. faecalis* isolate was inoculated in plates with Muller-Hinton medium (Oxoid®, UK) impregnated with various antibiotic disks in different concentrations. Subsequently, the plates were incubated at 37°C for 18-24h. After this period, the inhibition halo formed around each disk was measured and registered.

3. Results

Enterococcus faecalis isolates showed a higher prevalence of rifampicin resistance (77.7%). Additionally, these isolates also demonstrated resistance to erythromycin (48.5%), ciprofloxacin (37.9%), and tetracycline (26.2%). For the remaining tested antibiotics, lower levels of resistance were observed (≤ 19.4%), being that, none of the isolates showed resistance to gentamicin and streptomycin. All *E. faecalis* isolates were intrinsically resistant to quinupristin/dalfopristin (Table 1).

Almost half of the isolates (47.6%) showed multidrug-resistance profile (Figure 1), where 23.3% showed resistance to 3 different antimicrobial classes, 6.8% to 4 and 17.5% to 5 or more classes. On the other hand, 17.5% of the isolates showed resistance to 1 antimicrobial class and 26.2% to 2, however, 8.7% did not show resistance to any antibiotic (Figure 2).

Table 1. Resistance to different antimicrobial classes detected in *Enterococcus faecalis* isolates.

Antibiotic agent	Antimicrobial class	Resistant isolates	
		Number	%
Ampicilin (AMP)	Penicilins	3	2.9
Vancomycin (VA)	Glycopeptides	3	2.9
Teicoplanin (TEC)	Glycopeptides	3	2.9
Tetracycline (TE)	Tetracyclines	27	26.2
Erythromycin (E)	Macrolides	50	48.5
Ciprofloxacin (CIP)	Fluoroquinolones	39	37.9
Chloramphenicol (C)	Phenicols	4	3.9

Quinupristin/ dalfopristin (QD)	Streptogramins	103	100*
Nitrofurantoin (F)	Nitrofurantoins	19	18.4
Rifampicin (RD)	Ansamycins	80	77.7
Fosfomycin (FOS)	Fosfoycins	7	6.8
Gentamicin (CN)	Aminoglicosides	0	0
Streptomycin (S)	Aminoglicosides	0	0
Linezolid (LNZ)	Oxazolidinones	20	19.4

*: intrinsic resistance.

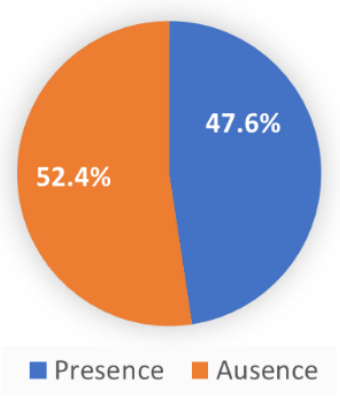

Figure 1. Percentage of the isolates with or no multidrug resistance (≥3 antibiotic classes) obtained in this study.

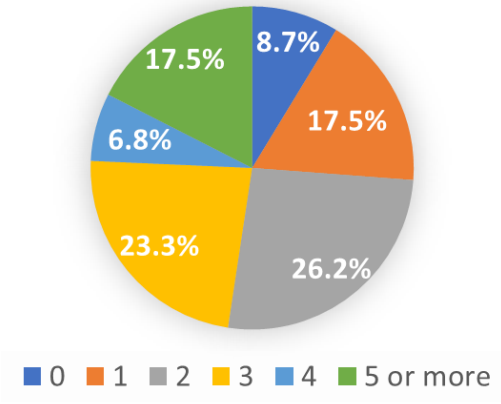

Figure 2. Percentage of the *Enterococci* isolates that showed resistance to antimicrobial classes analyzed in this study.

4. Discussion

Enterococci are commonly found in several animals and food of animal origin. However, the data described in the literature about resistance to antibiotics in ornamental animal feed, is still very scarce. In our study, the presence of *E. faecalis* was identified in 28 of 57 analyzed ornamental samples.

Recently, Dolka et al. [11] evaluated the presence of *Enterococcus* spp. in racing pigeons (*Columba livia f. Domestica*), observing that almost all isolates, about 93.1%, were resistant to at least one antibiotic and that *E. faecalis* was one of the most identified species in these birds. In this study, *E. faecalis* showed resistance more frequently to teicoplanin (100%) and erythromycin (82.4%). Another study whose aim was to determine antimicrobial susceptibility and virulence traits of *Enterococcus faecalis* isolates from human clinical specimens and retail red meat in Slovenia, it was observed that 29.6% of *E. faecalis* with a

clinical origin, showed multidrug resistance. It was also verified that the majority of clinical *E. faecalis* isolates were resistant to tetracycline (78.9%), followed by the resistance to erythromycin (46.5%) [12]. In comparison with Dolka et al. [11] and Golob et al. [12], our study showed that *E. faecalis* presented significant resistance values to erythromycin (48.5%), but it has a lower percentage of resistance to tetracycline (26.2) and teicoplanin (2.9%). However, the number of multiresistant isolates is higher in our study compared to that obtained in Golob et al. [12].

5. Conclusions

These results indicated a significant presence of *E. faecalis* in the feeding of ornamental animals, as well as, multidrug-resistant isolates, becoming a public health problem given the proximity and interaction of humans with these animals. Therefore, it is necessary to resort new alternatives to curb this problem, and the use of probiotics, phytochemicals, bacteriophages, among others, can be used as substitutes for antibiotics.

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Conflicts of Interest: The authors declare no conflict of interest.

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