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Occurrence and assessment of antibiotic resistance and virulence of *Enterococcus* spp. strains isolated from fecal samples of wild animals

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About 60% of the etiological agents of human infections are of animal origin, and the microorganisms causing them can be isolated not only from farmed and domestic animals, but also from wildlife. *Enterococcus* spp. may exhibit intrinsic or acquired resistance to many antibiotic groups, posing significant therapeutic challenges. The aim of this study was to identify and assess the antibiotic resistance and virulence genes of *Enterococcus* strains isolated from fecal samples of wild animals. The 118 strains were obtained from deer (n = 38), wild boar (n = 29), hare (n = 19), roe deer (n = 12), fallow deer (n = 5), raccoon dog (n = 4), fox (n = 4), moose (n = 2), polecat (n = 2), rabbit (n = 1), wolf (n = 1) and marten (n = 1). Antibiotic resistance assessments were performed using the disk diffusion method following the recommendations of the European Committee on Antimicrobial Susceptibility Testing (EUCAST). The frequency of occurrence of vancomycin-resistant enterococci (VRE) phenotypes, high-level streptomycin resistance (HLSR), high-level gentamicin resistance (HLGR), and high-level aminoglycoside resistance (HLAR) was also determined. The PCR was used to detect virulence genes (VGs) (*agg*, *gelE*, *EfaAfs*, *ace*, *pil*, *ebpA*, *ebpB*, *ebpC*, *srtA*, *hyl*, *asa*, *cylA* and *cylB*). The study revealed a high species diversity of *Enterococcus* spp. Among the 118 strains collected, 70 were resistant to at least one antibiotic. The majority of strains exhibited resistance to eravacycline, while the least resistance was observed against ampicillin. Strains with VRE, HLSR, HLGR, and HLAR phenotypes were identified. Multidrug resistant (MDR) strains were detected. However, extensively drug-resistant (XDR) and pandrug-resistant (PDR) strains were not observed. The virulence factors were present in the tested strains, and the most frequently detected gene was *agg* encoding aggregation substance. We have provided evidence that healthy wild animals can be reservoirs of pathogenic *Enterococcus* strains, including MDR strains and with many VGs, which can be transmitted to humans.

Keywords Wild animals, Feces, Pathogens, Zoonoses, *Enterococcus* spp., Antibiotic resistance, Virulence factor

Approximately 60% of human infectious diseases are zoonotic[1,2]. Pathogens causing infections in humans can be sourced from both wild and domesticated animals. In Poland, the most prevalent wild animals include roe deer, red deer, hares, and wild boars[3,4]. Various environmental changes resulting from human activities, such as habitat destruction, ongoing urbanization, and crossbreeding of domestic and wild animals, have led to an increased frequency of human contact with animals inhabiting wild areas, consequently elevating the importance of these animals in the spread of diseases[5].

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"One Health" is an initiative created and advocated by the World Health Organization (WHO). It is characterized by a balanced, integrated, and multisectoral approach to the health of humans, animals, and the environment. The main premise is the collaboration of public health, veterinary, ecological, and research sectors to predict, detect, and prevent global health threats. One of the primary actions defining the "One Health" initiative is the prevention, detection, and control of zoonotic diseases, as well as reducing the prevalence of Antimicrobial Resistance (AMR)[6].

The *Enterococcus* genus comprises Gram-positive, non-spore-forming cocci characterized by low nutritional requirements[7]. They exhibit high adaptability, including resistance to desiccation, the ability to grow in the presence of high salt concentrations, and a wide range of pH and temperature tolerance, making them widespread in the natural environment. *Enterococcus* spp. tolerate high concentrations of bile salts, allowing them to colonize the digestive tracts of many animal species, including humans[8]. The most commonly isolated species from mammals are *E. faecalis, E. faecium, E. hirae*, and *E. durans*[9]. *E. faecalis* and *E. faecium* are of greatest clinical significance, often causing hospital-acquired infections such as endocarditis, urinary tract infections, and infections of soft tissues and postoperative wounds[10]. Infections with *Enterococcus* spp. have also been observed in animals, causing diarrhea in livestock species such as pigs and cattle and urinary tract infections in domestic cats and dogs[11–13]. In recent years, there has been an increase in microbial resistance to antibiotics. The resistance of *Enterococcus* spp. to antimicrobial agents is linked to the acquisition of antibiotic resistance genes by these microorganisms[14].

The *Enterococcus* genus is characterized by intrinsic resistance to a broad group of β-lactam antibiotics, including cephalosporins. This is related to two specific penicillin-binding proteins (PBPs), Pbp4(5) and PbpA(2b), exhibit low reactivity toward cephalosporins, allowing these PBPs to cross-link peptidoglycan in the presence of cephalosporins. Moreover, the CroS/R two-component signal transduction system (TCS) is also required for cephalosporin resistance. However, the specific genes regulated by CroS/R that are responsible for these resistance has not yet been fully characterized[15–17]. These bacteria also exhibit reduced susceptibility to penicillins[18,19], especially among clinical *E. faecium* strains. Penicillin resistance in *E. faecalis* is due to Pbp4(5) overproduction and/or mutations[20]. Vancomycin-resistant enterococci (VRE) pose a particular threat by producing a different structure of peptidoglycan precursors, a component of bacterial cell walls[14]. High-level resistance to aminoglycosides (HLAR) is encoded by genes that modify the antibiotic. The HLGR phenotype (high-level gentamicin resistance) is characterized by a high level of resistance to all aminoglycosides, except streptomycin. High-level streptomycin resistance (HLSR) is reported as resistance only for streptomycin[19,21,22].

Enterococcus virulence is associated with many virulence genes (VGs), of which two main groups can be distinguished: secreted virulence factors such as cytolysin (*cylA*), gelatinase (*gelE*) and hyaluronidase (*hyl*), which damage host tissues, and those associated with aggregation to the cell surface, such as aggregating substances (*asa1*), enterococcal surface protein (*esp*), endocarditis antigen (*efaA*) and collagen-binding protein (*ace*). Additionally, expression of pili (encoded by the *ebpABC*, *srt*, and *pil* locus) on the cell surface helps adhesion and biofilm formation[23–26]. In addition to antibiotic resistance, monitoring the occurrence of virulence factors among *Enterococcus* strains isolated from the natural environment is important in the context of the "One Health" trend.

The aim of this study was to identify and assess the antibiotic resistance and selected virulence genes of *Enterococcus* spp. strains isolated from fecal samples of wild animals.

Material and methods

Origin and collection of samples

Fecal samples from wild animals were collected from forested areas and ecotone zones in two forestry districts in the Kuyavian-Pomeranian Voivodeship, Poland (Table 1). Only fresh fecal samples were collected during the study. The freshness of the samples was assessed based on criteria such as color, sheen and consistency. The species identification of the animal from which the feces originated was determined by an experienced and long-

Species of animal	Number of fecal samples (n = 98) (%)	Number of isolated strains (n = 118)
Deer (*Cervos*)	31 (31.6)	38
Hare (*Lepussaxatilis*)	18 (18.4)	19
Wild boar (*Sus scrofa*)	17 (17.0)	29
Roe deer (*Capreoluscapreolus*)	15 (15.3)	12
Fox (*Vulpes*)	4 (4.1)	4
Fallow deer (*Dama dama*)	3 (3.1)	5
Moose (*Alcesalces*)	3 (3.1)	2
Raccoon dog (*Nyctereutesprocyonoides*)	3 (3.1)	4
Wolf (*Wolf*)	1 (1.0)	1
Rabbit (*Oryctolaguscuniculus*)	1 (1.0)	1
Marten (*Murinisthorace*)	1 (1.0)	1
Polecat (*Mustelaputorius*)	1 (1.0)	2

Table 1. List of wild animal species, along with the number of fecal samples and isolates collected from each species.

serving forestry employee. Fecal fragments were collected manually and transferred to sterile, appropriately labeled containers, which were then transported to the laboratory within approximately 1 h of collection.

Isolation of *Enterococcus* spp. from samples

All samples were mechanically homogenized, and approximately 0.5 g aliquots were placed in tubes containing 4.5 ml Brain Heart Infusion (BHI) broth (Becton–Dickinson) and incubated at 37 °C for 24 h. After incubation in BHI (Becton–Dickinson), 0.5 ml of the mixture was transferred to a broth with sodium azide (Merck) (4.5 ml). The tubes were then incubated at 37 °C for 24 h. After incubation, 20 µl of the mixture was inoculated onto Enterococcosel Agar (Becton–Dickinson). The samples were incubated at 37 °C for 24 h. All presumptive *Enterococcus* colonies were select for identification.

Isolates identification

Species identification was performed using the Matrix-Assisted Laser Desorption/Ionization, Time of Flight (MALDI-TOF) system—Microflex (Bruker) according to the manufacturer's instructions. To preserve the research material, single colonies of identified microorganisms were transferred to Eppendorf tubes with BHI broth (Becton–Dickinson) and 15.0% glycerol (Avantor) and frozen at − 80 °C.

Assessment of *Enterococcus* spp. strains susceptibility to antibiotics

The antibiotic susceptibility was assessed using the disk diffusion method with 14 antibiotics: streptomycin (300 µg), ampicillin (2 µg), tigecycline (15 µg), norfloxacin (10 µg), gentamicin (30 µg), teicoplanin (30 µg), eravacycline (20 µg), dalfopristin-quinupristin (15 µg), vancomycin (5 µg), nitrofurantoin (100 µg), levofloxacin (5 µg), ciprofloxacin (5 µg), linezolid (10 µg) and imipenem (10 µg) (Argenta). Susceptibility assessments for nitrofurantoin were only performed for *E. faecalis*, for dalfopristin-quinupristin only for *E. faecium*. The zones of growth inhibition, the presence of VRE, HLGR (high-level gentamicin resistance), HLSR (high-level streptomycin resistance), and HLAR (high-level aminoglycoside resistance) phenotypes were determined according to the EUCAST (European Committee on Antimicrobial Susceptibility Testing) v. 13.0 recommendations[27]. Vancomycin resistance was confirmed by determining the minimum inhibitory concentration (MIC) using MIC Test Strips (Liofilchem). The HLSR phenotype was identified when resistance to streptomycin was detected, HLGR in the case of gentamicin resistance, and the HLAR phenotype was identified in strains resistant to both gentamicin and streptomycin.

The plates were incubated at 35 °C for 18 ± 2 h. After the incubation period, the zone of inhibition around the antibiotic disks were measured. *E. faecalis* ATCC 29212 was used as a control.

Classification of strains (multidrug-resistant, extensively drug-resistant, pandrug-resistant)

The classification of each strain into three groups: multidrug-resistant (MDR), extensively drug-resistant (XDR), and pandrug-resistant (PDR), and thus the determination of the degree of resistance, was based on the guidelines established by Sweeney et al.[28]. Briefly, a strain was considered MDR when it was resistant to at least one antibiotic in three or more classes. Strain was XDR, when it was resistance to at least one antibiotics in all classes, expected for one or two. Finally, strain was PDR, when it was resistant to all available antibiotics from all chemical groups[28].

Detection of virulence genes (VGs)

To detect VGs, isolation of DNA and multiplex PCR reactions were performed. DNA from the *Enterococcus* spp. strains was isolated using thermal method[29]. A single colony was suspended in 100 µl of 1 × Tris–EDTA buffer (pH 8.0) (Sigma-Aldrich). Next, it was incubated at 90 °C for 10 min. After this time, the mixture was cooled on ice for 2 min, and then the samples were centrifuged for 5 min (16,000×g, 4 °C). Purified DNA was placed in new tubes and stored at − 20 °C until further studies. The presence of genes encoding the following VFs: endocarditis and biofilm-associated pili (*ebpA, ebpB, ebpC*), pili (*pil*), pilus-associated sortase (*srt*), collagen-binding protein (*ace*), aggregation substance (*agg, asa1*), gelatinase (*gelE*), hyaluronidase (*hyl*), *E. faecalis* specific endocarditis antigen (*EfaAfs*), cytolysin activator (*cylA*), transport of cytolysin (*cylB*) was detected using a multiplex PCR, according to the Stępień-Pyśniak et al.[30] with some modification (Supplementary Table S1 online). The *E. faecalis* ATCC 29212 strain was used as positive control. As a negative control, a reaction mixture without DNA was used.

Results

The occurrence of *Enterococcus* spp. in fecal samples of wild animals

A total of 98 fecal samples from 12 species of animals were examined (Table 1). Enterococci were isolated from 92 (93.9%) samples, resulting in 118 strains belonging to nine species (Table 2). Some samples contained multiple isolates. Among the species, *E. faecalis* was the most frequently identified (38 strains; 38.2%), followed by the clinically significant E. *faecium* (7 strains; 7.6%). The least commonly detected species were *E. avium* and *E. thailandicus*, with one strain each (0.9%) (Table 2). The samples from deer showed the highest diversity of *Enterococcus* species, likely reflecting the larger number of fecal samples analyzed from this group (n = 31). Seven species were identified from deer, with *E. hirae* being the most common (26.0%) and *E. avium* the least frequent (2.0%). From rabbit feces (n = 1), only *E. mundtii* was identified, while *E. faecium* was the sole isolate from wolf feces (n = 1). Polecat feces (n = 1) yielded two species: *E. mundtii* and *E. casseliflavus*. For wild boars (n = 17), *E. hirae* was the most prevalent species (21.0%) (Table 1).

Enterococcus species	Number of strains (n = 118) (%)
faecalis	38 (32.2)
hirae	22 (18.6)
mundtii	19 (16.1)
casseliflavus	17 (14.4)
faecium	9 (7.6)
gallinarum	6 (5.1)
durans	5 (4.2)
avium	1 (0.9)
thailandicus	1 (0.9)

Table 2. Species composition of the *Enterococcus* population obtained from wild animals along with the number of isolates.

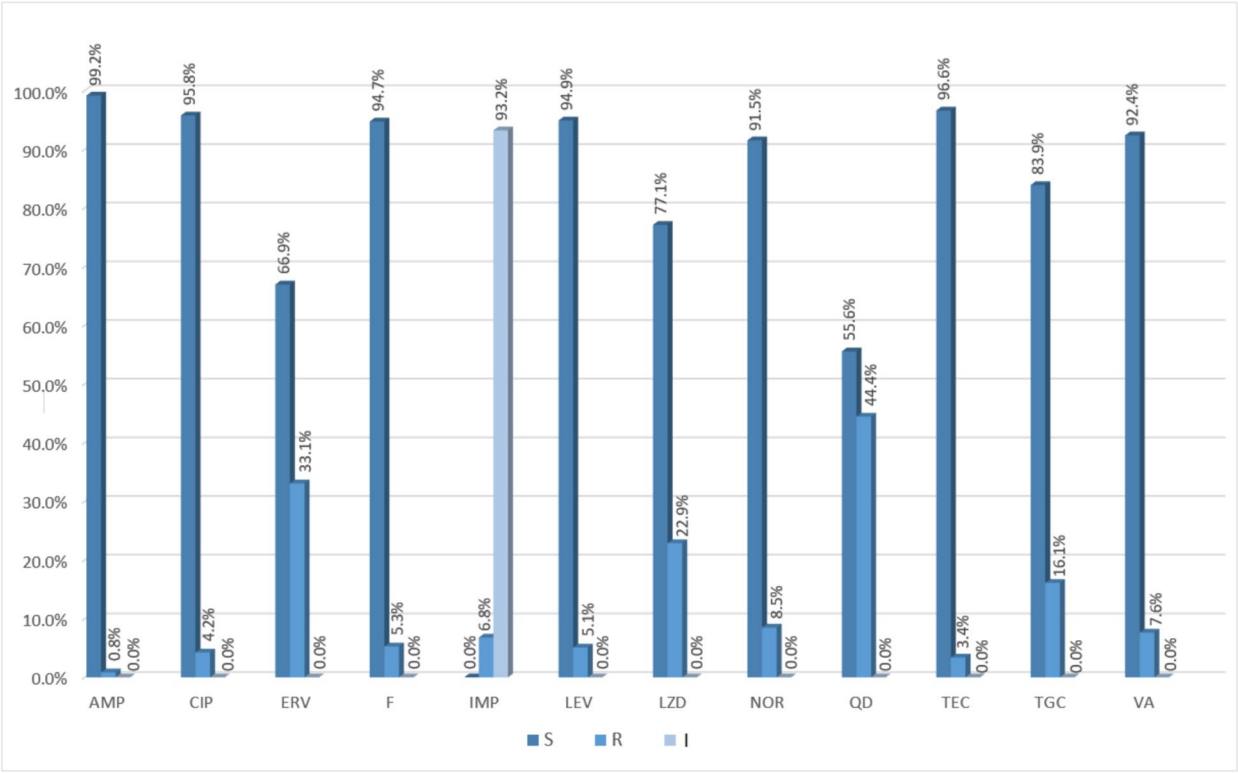

Fig. 1. Susceptibility of *Enterococcus* spp. (n = 118) strains to antibiotics. AMP—ampiciline, CIP—ciprofloxacin, ERV—eravacycline, F—nitrofurantoin, IMP—imipenem, LEV—levofloxacin, LZD—linezolid, NOR—norfloxacin, QD—quinupristin-dalfopristin, TEC—teicoplanin, TGC—tigecycline, VA—vancomycin, I- susceptible, increased exposure, R—resistant, S—susceptible.

Assessment of *Enterococcus* spp. strains susceptibility to antibiotics

Among the 118 tested strains, 70 (59.3%) were resistant to at least one antibiotic (Fig. 1). Resistance to eravacycline was most common (33.1%; 39 strains), whereas resistance to ampicillin was rare (0.8%; 1 strain). The highest percentage of strains resistant to antibiotics from different chemical groups was observed among *E. faecalis* and *E. faecium*, and the lowest among *E. avium* and *E. thailandicus*.

For *E. faecalis* (n = 38), most strains were resistant to eravacycline (50.0%), while resistance to nitrofurantoin, imipenem, and norfloxacin was low (5.3% each). In *E. hirae* (n = 22), resistance to eravacycline was also dominant (31.8%), with resistance to imipenem and norfloxacin limited to one strain each (4.6%). For *E. mundtii* (n = 19) and *E. casseliflavus* (n = 17), also most strains were resistance to eravacycline. Notably, *E. thailandicus* showed no resistance to any of the antibiotics, while the one strain of *E. avium* showed resistance only to eravacycline.

Resistance was identified in strains from 9 of the 12 animal species examined. No resistant strains were found in moose, rabbit, or wolf samples (Table 3).

Species of animal	Antibiotic	Percentage of resistant strains (%)
Deer	Eravacycline	39.5
Fallowdeer	Norfloxacin	80.0
Roedeer	Gentamicin	33.3
	Linezolid	33.3
	Eravacycline	33.3
Moose	–	–
Hare	Eravacycline	36.8
Rabbit	–	–
Fox	Tigecycline	50.0
Wolf	–	–
Raccoon dog	Ampicillin	25.0
	Norfloxacin	25.0
	Nitrofurantoin	25.0
	Levofloxacin	25.0
	Ciprofloxacin	25.0
	Imipenem	25.0
	Eravacycline	25.0
Marten	Tigecycline	100.0
	Gentamicin	100.0
	Linezolid	100.0
	Eravacycline	100.0
Polecat	Norfloxacin	50.0
	Vancomycin	50.0
	Eravacycline	50.0
Wild boar	Linezolid	27.6

Table 3. A list of antibiotics to which strains isolated from particular animal species most often showed resistance.

Detection of phenotypes: VRE, HLAR, HLSR, HLGR

The VRE phenotype was detected in two (1.7%) strains of *E. faecalis* isolated from roe deer and wild boar feces. The HLSR phenotype was detected in six (5.1%) of all strains (three *E. hirae*, one each of *E. durans*, *E. casseliflavus*, *E. faecalis*). Meanwhile, HLGR was observed in 10 strains (five *E. durans*, three *E. hirae*, one each of *E. casseliflavus* and *E. faecalis*). The highest percentage of strains resistant to streptomycin occurred in hares—two (10.5%), wild boars—two (6.9%), and deer—two (5.3%).

HLAR phenotype was demonstrated in five (4.2%) of all strains. This phenotype was detected in one (20.0%) strain of *E. durans*, three (13.6%) strains of *E. hirae*, and one (2.6%) strains of *E. faecalis*. The presence of the HLAR phenotype was detected in two (5.3%) strains from deer, two (6.9%) strains from wild boars, and one (5.3%) strain isolated from a hare.

Antibiotic resistance profiles

A total of 41 different resistance profiles were observed (Fig. 2). The most common profiles indicated susceptibility to imipenem with increased exposure (profile I, 41.5%), resistant to gentamicin, susceptible, increased exposure to imipenem (profile II, 6.8%), and III resistant to eravacycline; susceptible, increased exposure to imipenem (profile III, 5.1%). These profiles were most common in *Enterococcus* spp. isolates from deer, hare, and wild boar samples. The strain of *E. faecalis* isolated from roe deer feces exhibited resistance to seven antibiotics (profile XXXII). Overall, MDR was detected in 22.9% of strains, of which most strains were isolated from deer (18.4%). No PDR or XDR strains were found.

Prevalence of virulence genes (VGs)

Among the virulence genes (VGs) tested, *agg*, encoding aggregation substance, was the most common, found in 64.7% of strains (Table 4). Also, *hyl* and *gelE* genes were frequently detected in tested strains, at 43.7% and 42.0%, respectively. Other VGs, including *ebpC, srt*, *EfaAfs ace, ebpB, ebpA*, were present in 16.0–30.0% of strains, whereas *cylA* and *cylB* were absent in all isolates (Table 4).

The greatest number of VGs were found in *E. faecalis* strains. The tested strains were classified into 35 different virulence profiles (Table 5). The largest number of strains had the *agg* and *hyl* genes (20; 16.8%) and the *agg* gene (18; 15.1%). Only in nine (7.6%) strains none of the tested VGs were detected.

Discussion

Wild animals serve as a natural reservoir of microorganisms, some of which may prove to be potentially pathogenic not only for themselves but also for domestic animals and humans. The significant increase in the

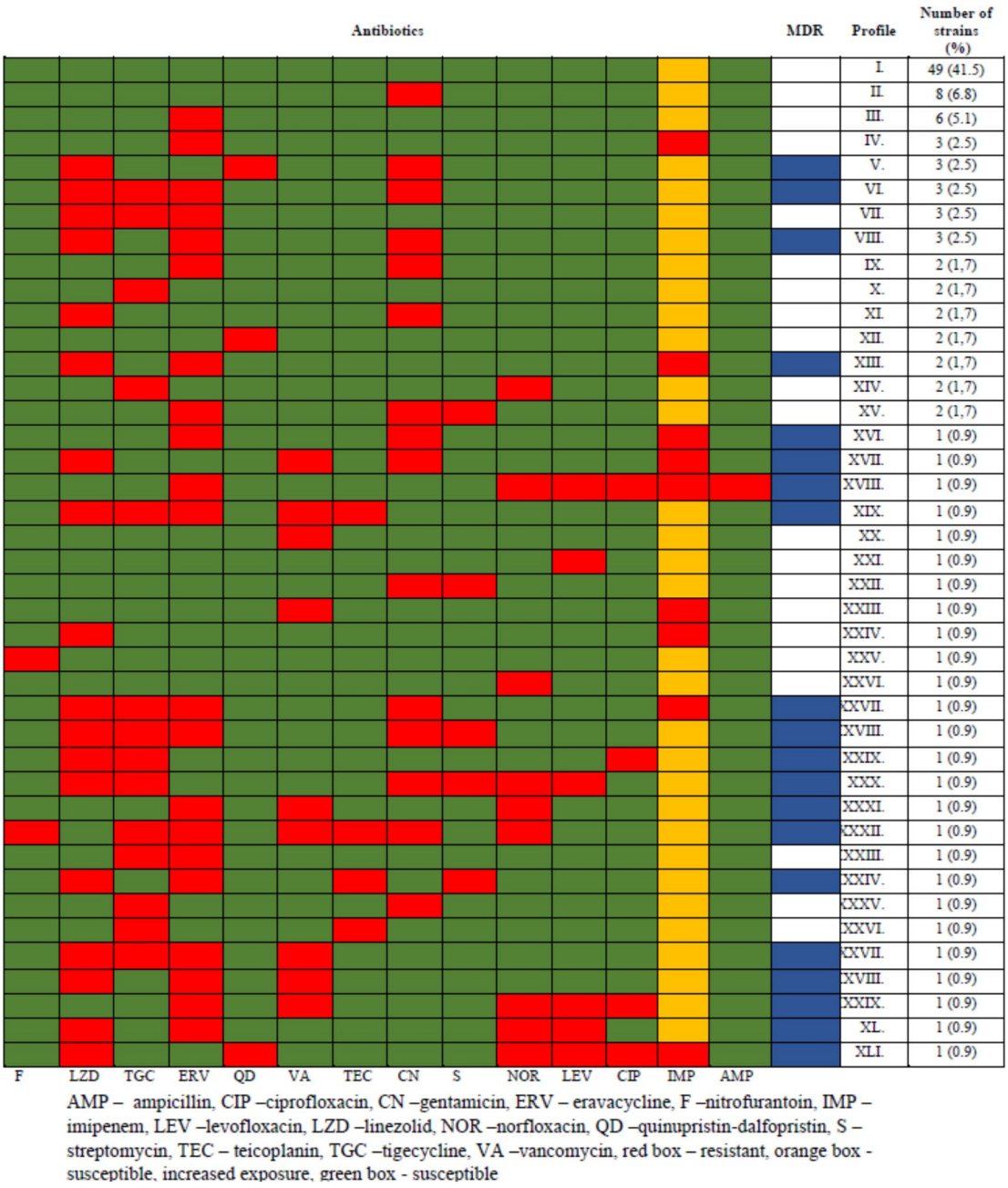

Fig. 2. Heatmap with antibiotic resistance profiles of *Enterococcus* spp. strains.

transfer of microorganisms, including pathogens, between these two niches has been observed due to human economic activities and the urbanization of forested areas. There are several diseases that can be transmitted between animals and humans through direct contact with an infected individual, as well as through contact with their feces or consumption of animal products[31–33]. The increasing prevalence of bacterial resistance to antibiotics is also becoming a significant problem[34,35].

The aim of the study was to identify and evaluate the antibiotic resistance of *Enterococcus* spp. strains that could be a zoonotic pathogenic factor. A total of 12 species of animals (98 collected samples) were examined, resulting in the isolation of nine species of enterococci (118 strains). The occurrence of enterococci in wild animals is not well-described in the literature, as it is in the case of farm animals such as poultry. There is limited research on *Enterococcus* spp. strains isolated from wild animals.

In this study, the number of fecal samples from which *Enterococcus* spp. strains were isolated was 92 (93.9%). In a study conducted by Cagnoli et al.[36] on Italian wild avifauna species, *Enterococcus* spp. were isolated from all 103 fecal samples. In a study conducted by Kemper et al.[37] on semi-domesticated cervid populations, enterococci were isolated from 92.9% (2224 out of 2243) of samples. Similar results were obtained by Dias et al.[34], who recovered *Enterococcus* spp. isolates from 89.0% of the Red Fox (*Vulpes vulpes*) fecal samples.

Gene	Number of strains									Total no. (%)
	EFA	EHI	EMU	ECA	EFM	EGA	EDU	EAV	ETH	
agg	18	20	11	11	0	2	5	1	1	77 (64.7)
hyl	10	18	8	6	5	4	1	0	0	52 (43.7)
gelE	36	12	1	0	0	1	0	0	0	50 (42.0)
ebpC	35	0	0	0	0	0	1	0	0	36 (30.3)
srt	32	1	0	0	0	0	1	0	0	34 (28.6)
EfaAfs	28	0	0	0	0	0	0	0	0	28 (23.5)
ace	14	1	2	4	0	0	2	0	0	23 (19.3)
ebpB	22	0	0	0	1	0	0	0	0	23 (19.3)
ebpA	19	0	0	0	0	0	0	0	0	19 (16.0)
pil	8	0	0	0	0	0	0	0	0	8 (6.7)
asa	4	0	0	0	0	0	0	0	0	4 (3.4)
cylA	0	0	0	0	0	0	0	0	0	0 (0.0)
cylB	0	0	0	0	0	0	0	0	0	0 (0.0)

Table 4. Prevalence of VGs of *Enterococcus* spp. strains. EFA, *Enterococcus faecalis*; EHI, *Enterococcus hirae*; EMU, *Enterococcus mundtii*; ECA, *Enterococcus casseliflavus*; EGA, *Enterococcus gallinarum;* EDU, *Enterococcus durans;* EAV, *Enterococcus avium*; ETH, *Enterococcus thailandicus.*

The most clinically relevant species of the *Enterococcus* genus are *E. faecalis* and *E. faecium*[24,38]. In our study, *E. faecalis* was the most frequently isolated species among the obtained enterococci. On the other hand, *E. faecium* was isolated from 7.6% of samples. In a similar study conducted by García et al.[39], *E. faecalis* was isolated in 37.6%, and *E. faecium* in 17.5%. These authors also used MALDI TOF MS for identification similarly to ours. We obtained the highest number of strains from deer, which is related to the largest number of deer fecal samples included in our study. Nine species of *Enterococcus* bacteria were isolated from deer fecal samples, with *E. hirae* (26.0%) and *E. mundtii* (21.0%) being the most prevalent. In comparison, Liliehaug et al.[40] isolated only five strains of *E. faecalis* and three strains of *E. faecium* from 50 fecal samples from red deer. For wild boar feces, they reported a much higher percentage of *E. faecalis* at 93.8%. But they identified enterococci based on PCR. Concerning samples from foxes in our study, the presence of three *Enterococcus* spp. was detected, with *E. faecium* constituting 50.0% of all strains of this genus and *E. faecalis* 25.0%. In a study by Dias et al.[34], *E. faecalis* constituted 49.0% of all *Enterococcus* species, which is a higher percentage than in our study. In the same study, *E. faecium* was isolated less frequently at 39.0%, but this result is lower than in our study[34]. The differences may be related to the different identification method used by the authors who used PCR. In another study conducted in Brazil with 50 strains isolated from wild foxes, identified using MALDI-TOF MS 64.0% *E. faecalis* and 22.0% *E. faecium*[41]. For other animals, species diversity was lower than for deer or foxes in our study.

In our study, it was demonstrated that 59.3% (70/118) of the tested *Enterococcus* spp. strains were resistant to at least one antibiotic. In a study Lillehaug et al.[40], resistance to one or more antibiotics was found in all *Enterococcus* spp. strains isolated from cervids. Similarly, in a study Oliveira de Araujo et al.[41], 98.0% of all *Enterococcus* spp. strains isolated from foxes were resistant to at least one antibiotic. In our study, enterococci were least resistant to ampicillin (0.8%), a result that is low compared to the literature. In a study by Nocera et al.[33], as much as 75.0% of the tested strains were resistant to ampicillin. In another study, 7.2% of *Enterococcus* spp. strains were resistant to ampicillin, which is still a high result compared to our findings[39]. In our study, the highest percentage of strains resistant to antibiotics from different chemical groups was found among *E. faecalis* and *E. faecium*, and the lowest among *E. avium* and *E. thailandicus*.

The detection of resistance phenotypes (VRE, HLAR, HLGR, and HLSR) in *Enterococcus* spp. isolated from the feces of wild animals is a poorly understood issue, and there is a lack of relevant data in the available literature. However, databases contain studies related to farm animals. In an experiment conducted by Kim et al.[21], out of 345 strains of *Enterococcus* bacteria, 8.7% of the strains exhibited the HLAR phenotype. In our study, this percentage was 4.2% of the tested strains. In this study, the VRE phenotype was detected in only two out of 38 *E. faecalis* samples (5.3%), which constitutes 1.7% of all *Enterococcus* spp. strains. Dec et al.[38] obtained three out of 52 isolates with high level of resistance (minimal inhibitory concentration ≥ 1,024 µg/mL) to vancomycin and teicoplanin. Other authors[39,41] did not detect vancomycin-resistant strains derived from wild animals.

Strains that are resistant to multiple antibiotics (MDR, PDR, and XDR) pose a particular danger and therapeutic challenge. Bacteria are increasingly acquiring resistance not only to one group of antibiotics but to several or even all groups. In our study, multidrug-resistance was detected in 22.9% of *Enterococcus* spp. strains. No PDR or XDR strains were found, but an *E. faecalis* strain from deer was resistant to at least one antibiotic from 5 different chemical groups. For comparison, in a study on wild birds conducted in Italy by Cagnoli et al.[36], as much as 77.0% of *Enterococcus* spp. strains exhibited multidrug-resistance, almost 20.0% were PDR, and about 3.0% were XDR. In our study, the percentage of MDR strains in *Enterococcus* spp. isolated from wild deer feces was 20.69% (6/29). In a study by Oliveira de Araujo et al.[41] on foxes in Brazil, 66.0% of strains exhibited multidrug-resistance. In the present study 13 different VGs were detected. The most frequently detected genes were *agg*, *hyl* and *gelE,* which play an important role in the pathogenesis process. Similar results were obtained by Pillay et al.[42], who detected the presence of the *gelE* gene in more than 50% of *Enterococcus* strains isolated from

Profile number	Virulence genes	Total no. (%)
I	*agg, hyl*	20 (16.8)
II	*agg*	18 (15.1)
III	*hyl*	10 (8.4)
IV	No VGs	9 (7.6)
V	*gelE, agg, hyl*	7 (5.9)
VI	*gelE, agg*	5 (4.2)
VII	*ace, agg*	4 (3.4)
VIII	*ace, agg, hyl*	3 (2.5)
IX	*EfaAfs, gelE, ebpA, ebpB, agg, srtA*	3 (2.5)
X	*EfaAfs, gelE, ebpA, ebpB, srtA*	3 (2.5)
XI	*EfaAfs, gelE, ebpA, ebpB, ebpC, srtA, hyl*	2 (1.7)
XII	*EfaAfs, gelE, pil, ebpB, ebpC, srtA*	2 (1.7)
XIII	*ace*	1 (0.8)
XIV	*ebpB, ace, agg, srtA*	1 (0.8)
XV	*agg, srtA, hyl*	1 (0.8)
XVI	*gelE*	1 (0.8)
XVII	*gelE, ace, agg, hyl*	1 (0.8)
XVIII	*ebpC, hyl*	1 (0.8)
XIX	*gelE, ebpB, ebpC*	1 (0.8)
XX	*EfaAfs, gelE, srtA*	1 (0.8)
XXI	*gelE, srtA, hyl*	1 (0.8)
XXII	*EfaAfs, gelE, ebpB, ace*	1 (0.8)
XXIII	*gelE, ebpB, agg, srtA*	1 (0.8)
XXIV	*EfaAfs, gelE, ebpB, srtA, hyl*	1 (0.8)
XXV	*EfaAfs, gelE, ebpB, ace, agg, srtA*	1 (0.8)
XXVI	*EfaAfs, gelE, ebpA, ebpB, ebpC, ace, srtA*	1 (0.8)
XXVII	*EfaAfs, gelE, ebpA, ebpB, ace, agg, srtA, asa*	1 (0.8)
XXVIII	*gelE, ebpB, ebpC, ace, agg, srtA*	1 (0.8)
XXIX	*EfaAfs, gelE, ebpA, ebpB, hyl*	1 (0.8)
XXX	*EfaAfs, gelE, ebpB, agg, srtA*	1 (0.8)
XXXI	*gelE, ebpA, ebpB, ebpC, agg, srtA, hyl*	1 (0.8)
XXXII	*EfaAfs, gelE, ebpB, ebpC, srtA*	1 (0.8)
XXXIII	*EfaAfs, gelE, ebpB, ebpC, ace, agg, srtA, hyl, asa*	1 (0.8)
XXXIV	*EfaAfs, gelE, ebpB, ebpC, ace, srtA*	1 (0.8)
XXXV	*EfaAfs, gelE, pil, ebpA, ebpB, ebpC, ace, srtA, hyl*	1 (0.8)
XXXVI	*EfaAfs, gelE, pil, ebpA, ebpB, ebpC, agg*	1 (0.8)
XXXVII	*EfaAfs, gelE, ebpA, ebpB, ebpC, agg, srtA*	1 (0.8)
XXXVIII	*gelE, ebpA, ebpB, ebpC, srtA*	1 (0.8)
XXXIX	*EfaAfs, gelE, pil, ebpB, ebpC, agg, srtA, hyl*	1 (0.8)
XL	*gelE, pil, ebpA, ebpB, ebpC, ace, srtA*	1 (0.8)
XLI	*ebpA, ebpB, ebpC, ace, agg, srtA, hyl*	1 (0.8)
XLII	*EfaAfs, gelE, pil, ebpB, ebpC, ace, agg, srtA, asa*	1 (0.8)
XLIII	*gelE, ebpB, ebpC, ace, agg, srtA, asa*	1 (0.8)
XLIV	*EfaAfs, gelE, ebpA, ebpB, ebpC, srtA*	1 (0.8)
XLV	*EfaAfs, gelE, pil, ebpB, ebpC, ace, agg*	1 (0.8)

Table 5. Profiles of VGs in *Enterococcus* spp. strains.

chicken cloacal samples. Until now, the presence of VGs in strains isolated from wild animals has not been well known. Our study significantly expands the state of knowledge in this area and proves that *Enterococcus* strains, especially *E. faecalis*, isolated from this material may contain a number of VGs.

To sum up, it can be stated that *Enterococcus* spp. strains identified in this study demonstrated a moderate level of antibiotic resistance and virulence compared to findings from other studies on wild animals. Approximately 59.3% of the strains were resistant to at least one antibiotic, which is lower than some studies, such as Lillehaug et al.[40] and Oliveira de Araujo et al.[41], where nearly all tested strains exhibited resistance to multiple antibiotics. Additionally, the prevalence of MDR strains in our study was 22.9%, considerably lower than the 66–77% MDR rates reported in other research. Virulence factors were detected in a significant proportion of strains, but the diversity and frequency were generally comparable or slightly lower than in studies focusing on wild avifauna

or other mammals. Overall, while this study highlights important public health risks, the strains studied appear less resistant and virulent than those reported in certain other contexts, particularly in environments with higher antibiotic pressure.

The findings of this study align with the One Health framework by illustrating the interconnectedness of human, animal, and environmental health through the investigation of antibiotic-resistant and virulent *Enterococcus* spp. strains in wild animals. They provide valuable insights into how wildlife serves as reservoirs of multidrug-resistant pathogens with zoonotic potential, thus emphasizing the need for integrated surveillance to prevent the spread of AMR. These results are critical for understanding the environmental reservoirs of AMR and for developing strategies to control zoonotic risks, directly supporting One Health's goal of collaborative, cross-sectoral approaches to global health challenges.

Conclusion

In all fecal samples from wild animals, the presence of potentially pathogenic bacteria from the *Enterococcus* genus was detected. This study revealed that 23.0% constituted MDR strains. The assessment of resistance to the applied antibiotics, as well as the low proportion of strains with VRE and HLAR phenotypes, indicates overall low antibiotic resistance among *Enterococcus* spp. strains isolated from fecal samples of wild animals. This work has contributed to enhancing the current understanding of the complexity and antibiotic resistance of the gut microbiota of wild animals. The results, particularly the surprisingly high percentage of strains resistant to eravacycline, may serve as a basis for further comprehensive analyses. The study shows the occurrence of many VGs among the tested strains, of which the gene encoding the aggregation substance was detected most frequently.

Data availability

The data sets used and/or analyzed during the current study available from the corresponding author on reasonable request.

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Declarations

Competing interests

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Additional information

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