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Bacteriology

Virulence genes and antimicrobial resistance in *Enterococcus* strains isolated from dogs and cats in Northeast China

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ABSTRACT. This study aimed to characterize the antimicrobial resistance and virulence of *Enterococcus* from dogs and cats in Northeast China and evaluate its zoonotic risk based on a total of 469 enterococci strains from 610 samples, including 238 strains of *E. faecium* and 128 strains of *E. faecalis*. The isolation rate from police dog samples was 93.79%, pet dog samples was 69.90% and pet cat samples was 76.67%. The differences in the prevalence of *E. faecalis* among different hosts were statistically significant ($P<0.05$). The assays showed that most of the virulence genes detected were existed in *E. faecalis* and police dogs carried the least number of virulence genes. The correlation between enterococcal surface protein (*esp*) and aggregation substance (*asa1*) was determined. Enterococci are most resistant to tetracycline and erythromycin, 68.92% of the isolates were classified as multiple drug resistant. Significant differences ($P<0.01$) were found between *E. faecium* and *E. faecalis* in the resistance rates of nine antimicrobials. Four positive and four negative correlations were found between virulence genes and antimicrobial resistance. The results show that *Enterococcus* colonization and excretion in dogs and cats were related to animal species and living environments. Some correlation between virulence factors and antimicrobial resistance was obtained. This study confirmed the presence of strains carrying multiple virulence factors and antimicrobial resistance at the same time, suggesting a public health risk for dogs and cats as reservoirs of enterococci.

KEYWORDS: antimicrobial resistance, cats, dogs, *Enterococcus*, virulence factor

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The *Enterococcus* spp. are Gram-positive opportunistic anaerobic bacteria that can be found in a variety of natural environments, including soil and water [4]. Enterococcal infections can cause several human illnesses, including urinary tract infections, sepsis and endocarditis. Enterococci have emerged as hospital-acquired pathogens because of their high resistance to antimicrobials [28], including cephalosporins, aminoglycosides and streptogramins [22]. The potential for enterococci to acquire antimicrobial resistance through plasmid and transposon transfer, chromosomal exchange or mutation poses a substantial barrier to therapeutic approaches [24] and considering the ability of enterococci to acquire and transfer resistance genes, animal hosts are likely donor sources [21].

In addition to antimicrobial resistance, enterococcal virulence contributes to illness development and different virulence factors can cause different pathogenesis. For example, gelatinase (*gelE*) and exoenzyme (*SprE*) play specific roles in mediating *E. faecalis* autolysis and biofilm formation [44], but enterococcal surface protein (*esp*) promotes upstream urinary tract infection [17]. The most frequently studied are *E. faecalis* and *E. faecium* where most putative virulence factors are usually present in *E. faecalis*, while only a few are present in *E. faecium* [47].

Apart from human infections, enterococci can cause many livestock infections [9, 27, 46], so there is concern about the possibility of enterococcal transmission between humans and animals. One study concluded that most of the enterococcal strains that infected patients were different from those in livestock, which suggested that those strains from livestock had limited sharing of resistance genes [19]. However, this limitation does not mean zero probability and contrary evidence is found in other studies. Enterococcal strains from humans have been isolated from dogs and pigs [8, 16] and another study confirmed that the distribution of enterococci

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isolated from humans and dogs was similar [50].

Companion animals, particularly dogs and cats, have evolved into close family members [38], so there is a risk that they can serve as a repository of bacteria and transmit them to humans because of their close intimate physical contact [5]. To investigate the potential risk of companion animals in the transmission of enterococci, this study identified antimicrobial resistance phenotypes and virulence factors of enterococci collected in northeastern China and compared the differences between hosts. The correlation between virulence factors and antimicrobials from these animals was also evaluated.

MATERIALS AND METHODS

Sample collection

Sampling procedures were approved by the Experimental Animal Welfare and Ethics Committee of Changchun Veterinary Research Institute, Chinese Academy of Agricultural Sciences. Feces or anal swabs were collected by qualified professionals using sterile swab sticks, between August and November 2021. The samples included 145 police dog samples from a base in Harbin, 345 pet dog samples and 120 pet cat samples from Changchun Pet Hospital. The swabs were placed in physiological saline containing 20% glycerol and stored in liquid nitrogen for a brief period before being transported to the laboratory. All police dogs are healthy. Some pet dogs and cats had concurrent disease not caused by enterococcus infection.

Strain isolation

Each sample was resuspended in saline and an appropriate amount of bacterial suspension was inoculated onto enterococcal agar (Qingdao Hope Bio-Technology Co., Ltd., Qingdao, China). After incubating at 37°C for 16 to 18 hr, suspicious *Enterococcus* spp. colonies were selected for continued purification culture.

Species identification

The small black *Enterococcus* spp. colonies were treated with gram staining, gram-positive spherical bacteria and underwent species and biochemical identification using a BD Phoenix™-100 automated identification system (Becton, Dickinson and Co., Franklin Lakes, NJ, USA) [57].

Polymerase chain reaction (PCR) was used for identification with analysis by enterococcal genus 16S ribosomal RNA gene sequence specific primers listed in Table 1, with reference to the conditions earlier [26] and *E. faecium* ATCC35667 was used as a positive control. The PCR reaction system was 25 µL in total consisting of 2 × 12.5 µL Taq Master Mix (Com Win Biotech Co., Ltd., Beijing, China), 0.5 µL each of 10 pmol/µL upstream and downstream primers, one µL DNA sample and finally supplemented with ultrapure water to 25 µL. The reaction conditions were pre-denaturation at 94°C for 2 min, denaturation at 94°C for 1 min, annealing at 56°C for 1 min, extension at 72°C for 1 min, all for 30 cycles then a final extension at 72°C for 5 min. The PCR was conducted in a Veriti™ 96-well Thermal Cycler (Applied Biosystem, Carlsbad, CA, USA).

Detection of virulence genes

The presence of enterococcal virulence genes, including cytolysin (*cylA*), *esp*, aggregation substance (*asa*1), *gelE*, hyaluronidase (*hyl*), endocardial antigens in *E. faecalis* (*efaAfs*) and *E. faecium* (*efaAfm*) and adhesin in collagen (*ace*) was investigated by PCR,

Table 1. Sequences of specific primers and virulence identification primers

Gene(s)	Primer sequence (5′ to 3′)	Size (bp)	Reference
ent	ent-F:AGCGCAGGCGGTTTCTTAA ent-R:CTCGTTGTACTTCCCATTGT	678	[26]
cylA	cyl-F:ACTCGGGGATTGATAGGC cyl-R:GCTGCTAAAGCTGCGCTT	688	[55]
esp	esp-F:AGATTTCATCTTTGATTCTTGG esp-R:AATTGATTCTTTAGCATCTGG	510	
asa1	asaI-F:GCACGCTATTACGAACTATGA asaI-R:TAAGAAAGAACATCACCACGA	375	
gelE	gelE-F:TATGACAATGCTTTTTGGGAT gelE-R:AGATGCACCCGAAATAATATA	213	
hyl	hyl-F:TATGGGTAATGCTGGTCG hyl-R:GTCCCTTGCTTCGTGTTT	220	This study
efaAfs	efaAfs-F:GACAGACCCTCACGAATA efaAfs-R:AGTTCATCATGCTGTAGTA	705	[30]
efaAfm	efaAfm-F:AACAGATCCGCATGAATA efaAfm-R:CATTTCATCATCTGATAGTA	735	
ace	ace-F:CAACCGAATGTGATAGAAA ace-R:GTAACGGACGATAAAGGA	411	This study

as shown in Table 1. The primers of *hyl* (GenBank accession number: WP_002399773) and *ace* (GenBank accession number: WP_010714416) were designed using Primer 3.0 (https://bioinfo.ut.ee/primer3-0.4.0), with the reaction conditions as described previously [30, 55]. Three different PCR reactions were standardized with one quadruple as *cylA-esp-asa1-gelE* and two duplex as *hyl-efaAfs* and *efaAfm-ace*.

The PCR reaction system was 25 µL as 2 × 12.5 µL Multiplex PCR Buffer, 0.125 µL Multiplex PCR enzyme mix (Takara Bio Inc., Kusatsu, Japan), 0.5 µL upstream and downstream 10 pmol/µL primers, 2 µL template and finally supplemented with ultrapure water to 25 µL. All reaction conditions were consistent as pre-denaturation at 95°C for 15 min, denaturation at 94°C for 1 min, annealing at 50°C for 1 min, extension at 72°C for 1 min, all for 30 cycles and final extension at 72°C for 10 min.

Antimicrobial susceptibility

Drug susceptibility testing of enterococcal isolates was performed using the BD Phoenix™-100 automated identification and susceptibility testing system. The panel included 11 classes of a total of 13 antimicrobials with amikacin, ampicillin, ciprofloxacin, erythromycin, gentamicin, linezolid, mupirocin, nitrofurantoin, quinupristin-dalfopristin, rifampin, teicoplanin, tetracycline and vancomycin all present. High-level gentamicin resistance enterococci (HLGR) and vancomycin-resistant enterococci (VRE) strains were screened simultaneously. Susceptibility results were interpreted using Clinical and Laboratory Standards Institute criteria (CLSI, 2020).

Statistical analyzes

Bar graphs were plotted using GraphPad prism version 8.0 software. The SPSS version 20.0 software was used for data statistics. Chi-square tests were used to evaluate the statistical significance of gene prevalence which was detected from different sites and animal species. Binary logistic regression analysis ($P<0.05$) was applied to assess the relationship between virulence genes detected and drug resistance in the presence or absence of genes, coded as 1 or 0, respectively.

RESULTS

Isolation and identification of Enterococci

In this study, 469 strains were confirmed as enterococci by biochemical identification as shown in Supplementary Table 1. The distribution was 238 *E. faecium* (50.75%), 128 *E. faecalis* (16.94%), 91 *E. hirae* (19.40%), 11 *E. gallinarum* (2.35%) and one *E. avium* (0.21%). The isolation rate for police dog samples was 93.79%, 69.90% for pet dog samples and 76.67% for pet cat samples.

Prevalent isolation rates of *E. faecalis* from different hosts were statistically significant, with the *P* values between police dogs and pet dogs, or police dogs and pet cats respectively less than 0.05 ($P=0.003$), while the differences were not significant for *E. faecium* ($P>0.05$) as seen in Fig. 1.

Virulence gene prevalence

The detection rate of virulence genes is presented in Fig. 2, where the virulence profile differed between *E. faecium* and *E. faecalis*. In *E. faecium*, only the *efaAfm* gene is present. The detection rates of *E. faecalis* virulence genes were 32 *cylA* (25.00%), 55 *esp* (42.97%), 78 *asa1* (60.94%), 66 *gelE* (51.56%), 43 *hyl* (33.59%), 109 *efaAfs* (85.16%), 21 *efaAfm* (16.41%) and 110 *ace* (85.94%). The specific virulence profiles statistics are displayed in Supplementary Table 2 and *E. faecalis* occupied 22 of these species. Virulence genes detected from different hosts were expressed at various levels, with the least virulence detected in police dogs and statistically significant results are shown in Table 2.

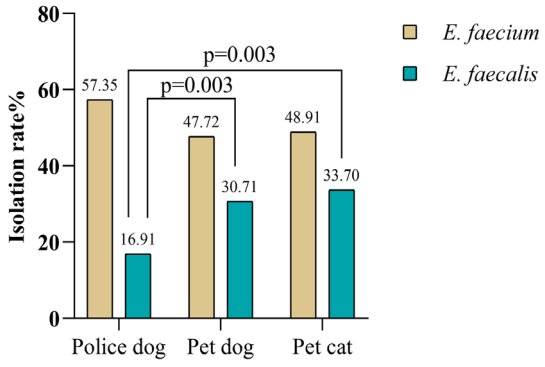

Fig. 1. Chi-square test and probability values for *Enterococcus faecium* and *E. faecalis*.

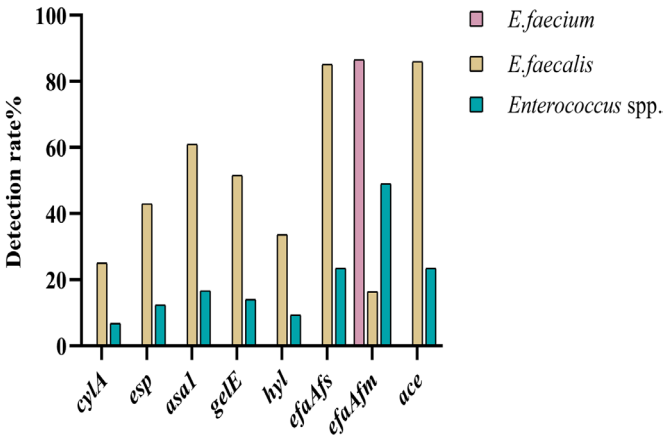

Fig. 2. Detection rate of enterococcal virulence genes. *Enterococcus faecium* (n=238), *E. faecalis* (n=128), *Enterococcus* spp. (n=469).

Table 2. Chi-square tests and probability values of virulence genes of different hosts

A	Police dog & Pet dog gene	χ^2	*P*
	cylA	4.562	0.033
	esp	11.961	0.001
	asaI	19.652	<0.001
	gelE	4.32	0.038
	efaAfs	7.556	0.006
	efaAfm	4.93	0.026
	ace	7.566	0.006
B	Police dog & Pet cat gene	χ^2	*P*
	cylA	17.5	<0.001
	esp	20.589	<0.001
	asaI	24.826	<0.001
	efaAfs	9.09	0.003
	ace	9.09	0.003
C	Pet dog & Pet cat gene	χ^2	*P*
	cylA	8.253	0.004

Table 3. Resistance rate of *Enterococcus faecium* and *E. faecalis* in different species

Antibiotics	*E. faecium*				*E. faecalis*				The other[a]	Total
	Total n=232[b]	Police dog n=78	Pet dog n=109[b]	Pet cat n=45	Total n=128	Police dog n=23	Pet dog n=74	Pet cat n=31	n=100	(n=460[b])
Amikacin[c]	73 (31.47)	22 (28.21)	19 (17.43)	32 (71.11)	79 (61.72)	19 (82.61)	32 (43.24)	28 (90.32)	10 (10.00)	162 (35.22)
Ampicillin[c]	66 (28.45)	15 (19.23)	23 (21.10)	28 (62.22)	3 (2.34)	0 (0.00)	3 (4.05)	0 (0.00)	0 (0.00)	69 (15.00)
Ciprofloxacin[c]	82 (35.34)	20 (25.64)	36 (33.03)	26 (57.78)	56 (43.75)	10 (43.48)	29 (39.19)	17 (54.84)	11 (11.00)	149 (32.39)
Erythromycin	159 (68.53)	50 (64.10)	69 (63.30)	40 (88.89)	85 (66.41)	10 (43.48)	50 (67.57)	25 (80.65)	44 (44.00)	288 (62.61)
Gentamicin[c]	69 (29.74)	17 (21.79)	20 (18.35)	32 (71.11)	66 (51.56)	7 (30.43)	36 (48.65)	23 (74.19)	9 (9.00)	144 (31.30)
Linezolid[c]	12 (5.17)	3 (3.85)	8 (7.34)	1 (2.22)	31 (24.22)	4 (17.39)	22 (29.73)	5 (16.13)	17 (17.00)	60 (13.04)
Mupirocin[c]	1 (0.43)	0 (0.00)	1 (0.92)	0 (0.00)	20 (15.63)	3 (13.04)	11 (14.86)	6 (19.35)	11 (11.00)	32 (6.96)
Nitrofurantoin[c]	121 (52.16)	29 (37.18)	63 (57.80)	29 (64.44)	11 (8.59)	2 (8.70)	9 (12.16)	0 (0.00)	25 (25.00)	157 (34.13)
Quinupristin-dalfopristin[c]	79 (34.05)	18 (23.08)	50 (45.87)	11 (24.44)	82 (64.06)	19 (82.61)	34 (45.95)	29 (93.55)	14 (14.00)	175 (38.04)
Rifampin[c]	118 (50.86)	21 (26.92)	75 (68.81)	22 (48.89)	96 (75.00)	17 (73.91)	55 (74.32)	24 (77.42)	8 (8.00)	222 (48.26)
Teicoplanin	3 (1.29)	0 (0.00)	3 (2.75)	0 (0.00)	4 (3.13)	0 (0.00)	4 (5.41)	0 (0.00)	8 (8.00)	15 (3.26)
Tetracyclin	193 (83.19)	77 (98.72)	83 (76.15)	33 (73.33)	101 (78.91)	16 (69.57)	60 (81.08)	25 (80.65)	86 (86.00)	380 (82.61)
Vancomycin	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (0.78)	0 (0.00)	1 (1.35)	0 (0.00)	1 (1.00)	2 (0.43)

Because of the small number of other *Enterococcus* spp. strains, only *E. faecium* and *E. faecalis* were shown in this table. a: Total enterococci other than *E. faecalis and E. faecalis.* b: Some strains could not be recognized by the BD system, so they were removed from the statistics. c: 9 kinds of antimicrobials with the significant difference in *E. faecium* and *E. faecalis.*

Antimicrobial susceptibility

Table 3 showed that the frequencies of resistance to tetracycline and erythromycin were higher, at 82.61% and 62.61% respectively. The resistance rate of eight species were between 10% and 50% with rifampin 48.26%, quinupristin-dalfopristin 38.04%, amikacin 35.22%, nitrofurantoin 34.13%, ciprofloxacin 32.39%, gentamicin 31.30%, ampicillin 15.00% and linezolid 13.04%. The low resistance rates included mupirocin at 6.96%, teicoplanin 3.26% and vancomycin 0.43%. There were significant differences ($P<0.01$) in the resistance rates of nine kinds of antimicrobials between *E. faecalis* and *E. faecium* (Table 3), including amikacin, ampicillin, ciprofloxacin, gentamicin, linezolid, mupirocin, nitrofurantoin, quinupristin-dalfopristinc and rifampin. Among isolates, 145 HLGR strains (31.52%) and four VRE strains (0.87%) were detected.

The antimicrobial resistance profile statistics are summarized in Supplementary Table 3. In total, 156 resistance profiles were detected against 13 antimicrobials. According to the current criteria for determining multidrug resistance (MDR) in bacteria [34], 68.92% of the isolates were classified as MDR and 98.70% were resistant to at least one class of antimicrobials.

Correlation between virulence gene distribution and drug resistance

Table 4 showed the relationship between the distribution of virulence genes and antimicrobials in *E. faecalis*. Eight distribution pairings were statistically significant ($P<0.05$). *E. faecalis* strains carrying the *asa1* gene were detected to be more resistant to

Table 4. The logistic regression analysis of antimicrobials and virulence genes in *Enterococcus faecalis*

Antibiotics	Genes	β	*P*	OR	OR95% (CI)
Gentamicin	*asa1*	2.144	0.001	8.532	2.439~29.844
	gelE	–1.673	0.011	0.188	0.052~0.684
Erythromycin	*asa1*	2.385	<0.001	10.856	3.018~39.049
	hyl	–1.742	0.013	0.175	0.045~0.690
Linezolid	*esp*	–1.93	0.022	0.145	0.028~0.759
Rifampin	*esp*	2.656	0.002	14.244	2.585~78.471
	gelE	–1.791	0.025	0.167	0.035~0.802
	hyl	1.629	0.034	5.1	1.135~22.919

OR, odds ratio; CI, confidence interval.

gentamicin and erythromycin. The same case for *esp* and *hyl* to rifampin. Conversely, *E. faecalis* strains carrying the *gelE* gene were detected to be more sensitive to gentamicin and rifampin, *hyl* to erythromycin, *esp* to linezolid in the same situation.

DISCUSSION

The bacterium *Enterococcus* was once considered a harmless commensal with probiotic properties which enhanced the immune system [41], but it has now become one of the most common pathogens of nosocomial infection [15]. Clinical enterococcal infections in companion animals are rare [52], but studies have proved that such pets can be reservoirs of enterococci [24–26]. A community-acquired case of multidrug resistant *E. faecalis* corneal ulcers caused by a pet cats scratch has been previously reported [39], so enterococci isolated from dogs and cats may cause cross-transfer of pathogenic bacteria [40]. A study demonstrates that enterococcal clones were found in pets in multiple body sites, their human cohabitants, and shared domestic objects [32], in which case regular monitoring of bacterial virulence genes and drug resistance can provide scientific guidance and timely interruption of the source of transmission.

The enterococcal microbiota of the intestinal tract of dogs and cats are predominantly *E. faecalis* and *E. faecium* [53] as shown in this study, where they were the main isolates. The isolation rates of *E. faecalis* in pet dogs and cats were significantly ($P<0.05$) different from those of police dogs. This meant that the prevalence of *E. faecalis* was statistically significant across hosts, while *E. faecium* was not. In a previous study, the effect of dog breeds on gut microbiomes was noted [33], which may explain the differences in isolation rates. In China, a police dog is a large breed working dog, while pet breeding is more varied according to the owner's preference. Analysis of the virulence genes showed that police dogs carried the lowest number and pet dogs and cats carried comparable levels. This result may reflect the influence of environment or exposure in the route of transmission, as police dogs live in a simple environment with a single population of contacts, but pets are frequently exposed to a variety of external environments and have closer contact with humans.

Virulence factors are determinants of infection-causing strains [54]. The *efaA* gene was the most frequently detected adhesin gene. In this study, *efaAfm* was found in 86.55% of *E. faecium* strains and *efaAfs* in 85.16% of *E. faecalis* strains. However, the presence of only *efaAfm* gene seems to have no value as a risk indicator in *E. faecium* strains [29]. The *E. faecium* and *E. faecalis* strains showed significantly different patterns in the incidence of virulence determinants and multiple virulence determinants were found in *E. faecalis* [11]. More attention has focused on *E. faecalis*, as most of the virulence factors in this study were found in these strains. The *ace* gene encoded a collagen-binding protein involved in the pathogenesis of endocarditis and its incidence rate was second only to *efaA*. The *esp* gene for enterococcal surface protein and the *asa1* gene for aggregation substance are associated with biofilm formation [13]. In the study, the detection rate of *esp* in *E. faecalis* was 42.97%, of which 94.55% also carried the *asa1* gene, demonstrating a strong association between the two genes.

The hyaluronidase encoded by the chromosomal *hyl* gene, is a degradative enzyme associated with tissue damage [35]. It has been shown that most *hyl* positive strains were also positive for *esp* in *E. faecium* [43]. The results suggested that the same conclusion does not exist for *E. faecalis*, with only 20.93% of strains matching this profile. The *gelE* gene encodes a zinc metalloprotease, with hydrolytic capacity. The results of this study indicated that 51.56% of enterococcal isolates were gelatinase producers. The *cylA* gene is a determinant of lysin production and enables the bacteria to evade the host immune response by destroying cells such as macrophages and neutrophils [56]. The detection rate of *cylA* was the lowest in this study, which is consistent with the results of a study related to human clinical infection [18].

This study found that 82.03% (105/128) of strains carried three and more virulence genes in *E. faecalis*. Based on the idea that the pathogenic potential may be due to virulence being multifactorial and associated with different genes, focusing on the role of individual virulence genes is no longer sufficient and the superposition of multiple virulence genes may pose new challenges for disease treatment [5].

Another factor affecting treatment after enterococcal infection is resistance to antimicrobials. A study has mentioned that tetracyclines and erythromycin belong to the class of antimicrobials commonly used in small animal veterinary medicine [20]. In China, there are no exact statistics on the use of antimicrobials in companion animals, but we found that tetracyclines and macrolides were among the

top sales by the China Veterinary Drug Association (http://www.cvda.org.cn/index.html). We speculate that the usage amount may be responsible for the high rate of tetracycline and erythromycin resistance in this study. In this study, 67.28% of the tetracycline resistant phenotypic strains were also resistant to erythromycin, which is consistent with the idea that enterococci tend to be resistant to both erythromycin and tetracycline antimicrobials [7].

Enterococci also have an acquired resistance to aminoglycosides and β-lactams. The resistance rate of *E. faecium* isolates was significantly higher for aminoglycosides, while ampicillin resistance was higher for *E. faecalis* isolates [36]. Compared with these results, it must be pointed out that although the rate of drug resistant strains was different, the results both confirmed the severity of drug resistance. This study showed that enterococcal strains had low levels of resistance to glycopeptide resistant drugs. Strains resistant to vancomycin and teicoplanin were assigned to *vanA* phenotype, while those susceptible to teicoplanin but resistant to vancomycin were considered as the *vanB* phenotype [49], but in this study, only one isolate was classified as *vanA*. In this study, nine kinds of antimicrobial susceptibilities differed between *E. faecium* and *E. faecalis*. The *E. faecalis* is inherently resistant to quinupristin-dalfopristin, which may explain the high rate of resistance to this antibiotic *E. faecalis*. For other antimicrobial agents in this experiment, until more conclusive evidence is available, the high resistance can only be attributed to the irregular use of antimicrobial drugs.

Evaluating risk factors that the presence of multi-drug resistant (MDR) *Enterococcus* strains in animals is crucial for public and environmental health [3, 6, 31]. In one study, 67.23% of *E. faecium* and 93.75% of *E. faecalis* isolates were found to be MDR strains [14], but contrary to the findings of Shahraki [48], this study found a higher rate of MDR in *E. faecalis*. The high rate of MDR results was not only present in companion animals but similar results were obtained in isolates from food, animals and the environment [10, 12, 14]. The current concern is that the acquired antimicrobial resistance among enterococci makes treatment very difficult [12], so it is crucial to monitor the resistance characteristics of isolates and regulate the use of antimicrobials in veterinary medicine.

The current study identified two types of resistant strains, where HLGR strains were found to exist in four species of enterococci. The HLGR not only affects the synergistic use of antimicrobials but has spread in hospitals [45], so its presence needs attention. The VRE strain which can lead to a more severe risk of infection, was found in only four strains and all of them were *E. faecalis*. Vancomycin is considered the last line of defense for enterococcal therapy and the advent of VRE presents an even greater challenge. The use of avoparcin was discontinued as a precautionary measure [37] and the study of linezolid and quinupristin-dalfopristin as alternative molecules for VRE infection [51] all aim to avoid the development of drug resistance, but they do not achieve complete blockage.

Exploring the correlation between resistance to antimicrobial agents and virulence factors of enterococcal strains can be of great significance for appropriate treatment initiatives by veterinary practitioners. One study suggested some correlation between antimicrobial resistance and different virulence determinants [2], which may increase morbidity and mortality [42]. In this study, a negative correlation between rifampicin and *gelE* was observed, which might be due to linezolid combined with rifampicin having a good therapeutic effect on biofilms [23]. In addition to the known significant association of the *esp* gene with resistance to ciprofloxacin, erythromycin, and tetracycline [1], it was also found that *esp* was related to linezolid and rifampicin resistance in this study. A definitive conclusion was not made and further studies are needed to examine the association of pathogenicity with multi-virulence and multi-drug resistance.

Virulence factors and drug resistance associated with plasmids were also considered. This has been described in other studies, as the plasmid addiction system, with the detection of transferable linezolid resistance genes (*optrA*) in enterococci and the facilitation of plasmid binding and exchange by aggregation substance [29, 41, 53]. Although such findings are still few, these studies deserve to be pursued in-depth as more specific routes of transmission.

In conclusion, this study found that *E. faecalis* in dogs and cats was more common and contained more virulence factors than *E. faecium,* with an association between virulence factors and antimicrobial resistance. Comparing isolation rates in enterococcal species, the results suggested that both the hosts and the environment influence the results of enterococcal harboring in dogs and cats. Based on the current results, more comprehensive surveillance of companion animals is recommended, as is the standardization of antimicrobial use in veterinary medicine to interrupt the possible transmission risk of *Enterococcus* spp. More studies should be conducted to find the cause of enterococcal pathogenicity.

CONFLICT OF INTEREST. The authors declare that they have no competing interests.

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