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Enterococci from Food-producing Animals at a Slaughterhouse: Antibiotic Resistance and Genetic Diversity in Turkey

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Enterococci from Food-Producing Animals at a Slaughterhouse: Antibiotic Resistance and Genetic Diversity in Turkey

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Abstract: Enterococci are widely acknowledged as prominent pathogens in hospitals, with the potential to transfer resistance genes, virulence factors, or other characteristics that increase their ability to survive in humans. Healthy cattle, sheep, and goats can be reservoirs for gastrointestinal pathogenic fecal enterococci, some of which could be multidrug-resistant to antimicrobials. The objective of this study was to determine the prevalence and diversity of *Enterococcus* species in healthy sheep, goat, and cattle carcasses, as well as to analyze the antimicrobial resistance phenotype/genotype and the virulence gene content. During 2019–2020, carcass surface samples of 150 ruminants were collected in a slaughterhouse. A total of 90 enterococci, comprising 5 species, were obtained. The overall prevalence of enterococci was found to be 60%, out of which 37.7% were identified as *E. hirae*, 33.3% as *E. casseliflavus*, 15.5% as *E. faecium*, 12.2% as *E. faecalis,* and 1.1% as *E. gallinarum*. Virulence-associated genes of *efaA* (12.2%) were commonly observed in the *Enterococcus* isolates, followed by *gelE* (3.3%), *asaI* (3.3%), and *ace* (2.2%). Characteristic resistance genes were identified by PCR with an incidence of 6.6%, 2.2%, 1.1%, 1.1%, 1.1%, and 1.1% for the *tetM*, *ermB*, *ermA*, *aac(6')Ie-aph(2")-la VanC1*, and *VanC2* genes, respectively. The high resistance to quinupristin-dalfopristin (28.8%), tetracycline (21.1%), ampisilin (20%), and rifampin (15.5%) was distributed among two, four, four, and five of the five species, respectively. The resistance of *Enterococcus* isolates to 11 antibiotic groups was determined and multidrug-resistant (MDR) strains occupied 18.8% of the *Enterococcus* isolates. Efflux pump genes causing multidrug resistance were detected in *Enterococcus* isolates (34.4%). The study's results showed that there were enterococci in the slaughterhouse that were multidrug resistant (MDR) and had a number of genes linked to virulence that could be harmful to human health.

Keywords: antibiotic resistance; food-producing animal carcass; *Enterococcus* spp.; MALDI-TOF MS; slaughterhouse

1. Introduction

Foodborne illnesses frequently occur after consuming contaminated food, particularly animal-derived products like meat [1]. Animal enterococci are easily found in animal-derived foods that are presumably frequently ingested by humans [2]. Enterococci are a component of the natural microbiota in the digestive systems of animals and humans, particularly *E. faecalis* and *E. faecium,* which have emerged as significant clinical concern. These nosocomial pathogens are recognized as the causative agents of various animal ailments, including mastitis, endocarditis, diarrhea, and septicemia in cattle, domesticated animals, swine, and poultry [3–5]. The species *E. durans*, *E. hirae*, *E. gallinarum*, *E. cassseliflavus*, *E. faecium*, and *E. faecalis* are frequently present in the gastrointestinal system of livestock [6].

Enterococci's pathogenesis is linked to a diverse range of virulence factors. Virulence factors contribute to the development of enterococcal infections by facilitating the attachment, colonization, and invasion of host tissues. They also affect the host's immune response and produce enzymes and toxins outside of cells, which worsen the severity of the illness. The key adhesion factors involved in biofilm development include Ebp (endocarditis and biofilm-associated pili), Asa (aggregation

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substance), EfaA (*E. faecalis* antigen A), Esp (extracellular surface protein), Ace (collagen-binding cell wall protein), cylA (hemolysin), efm (*E. faecium*-specific cell wall adhesin), cad1 (pheromone cAD1 precursor lipoprotein), sagA (secreted antigen), and cpd1 (pheromone cPD1 lipoprotein) [7–9].

The utilization of antimicrobials in animals is associated with the emergence of antimicrobial resistance, and mechanisms of antibiotic resistance can readily disseminate among microbial communities [10,11]. Both clinical and animal enterococci possess intrinsic resistance to several antimicrobial drugs, and they also have the ability to develop resistance to additional antimicrobial agents, such as glycopeptides, quinolones, tetracyclines, macrolides, and streptogramins [1,12,13]. While food-producing animals may not always directly transmit enterococci to humans, they can nonetheless facilitate the transfer of resistance genes from these animals to human germs. Hence, the occurrence of resistant enterococci, particularly vancomycin-resistant enterococci, in animals used for food production has emerged as a significant issue [12].

The emergence of antimicrobial resistance in zoonotic bacteria poses a substantial risk to public health, mainly due to the heightened likelihood of treatment failures. Furthermore, the emergence of resistance, particularly through the acquisition of transmissible genetic components, might also impact other characteristics, such as the capacity to inhabit an animal host or endure in an agricultural or food processing setting [14,15]. The presence of antibiotic-resistant enterococci in meat, animal-related sources, and habitats linked to animals, food handling equipment, and healthy humans emphasizes the importance of evaluating enterococci in slaughterhouses as well. The main place where zoonotic pathogens come from is the gastrointestinal tract (GI tract) of healthy food animals. Most food-related diseases are spread by feces during the slaughtering process or by cross-contamination during processing [14,16]. The transfer of harmful microorganisms from one part of the animal's body to another during the slaughtering process poses a substantial risk to the safety of the meat. Carcass tissues primarily become contaminated with fecal particles during the evisceration and skinning processes [17–19]. This study aimed to assess and characterize the prevalence, types, virulence determinants, and antimicrobial resistance profiles of enterococci from healthy cattle, sheep, and goat carcasses to highlight their zoonotic importance.

2. Results

2.1. Prevalence of Enterococci

The overall prevalence of enterococci in sheep, goat, and cattle animals from a slaughterhouse in Van, Turkey, was 60% (90/150). The species distribution is shown in Table 1. The predominant species evaluated were *E. hirae* (n = 34, 37.7%) and *E. casseliflavus* (n = 30, 33.3%). A smaller number of *E. faecium* (n = 14, 15.5%), *E. faecalis* (n = 11, 12.2%), and *E. gallinarum* (n = 1, 1.1%) were also evaluated. The number of *Enterococcus* species in the brisket, flank, hind leg, and rectal sides of carcasses is shown in Table 3. *Enterococcus* species contamination was not significantly different in animal species in comparison to carcass sites ($P < 0.05$). *E. casseliflavus* and *E. faecium* were isolated from 10 and 6 out of 150 carcass surface samples from the brisket and hind leg, respectively, whereas *E. hirae*, *E. faecalis*, and *E. gallinarum* were isolated from 13, 5, and 1 out of 150 carcass surface samples from rectal swabs, respectively.

Table 1. Prevalence of *Enterococci* in brisket, flank, hind leg, and rectal swab samples.

Animal species	Carcass surface point	Sample number	*E. faecalis*	*E. hirae*	*E. faecium*	*E. casseliflavus*	*E. gallinarum*	Total
			n(%)	n(%)	n(%)	n(%)	n(%)	n(%)
Sheep	Brisket	80	0(0)	4(11.7)	0(0)	7(23.3)	0(0)	11(12.2)
	Flank	80	1(9.09)	5(14.7)	2(14.2)	1(3.3)	0(0)	9(10)
	Hind leg	80	2(18.1)	4(11.7)	6(42.8)	4(13.3)	0(0)	16(17.7)
	Rectal	80	4(36.3)	8(23.5)	2(14.2)	4(13.3)	0(0)	18(20)
Goat	Brisket	50	1(9.09)	1(2.9)	0(0)	3(10)	0(0)	5(5.5)
	Flank	50	0(0)	1(2.9)	1(7.1)	2(6.6)	0(0)	4(4.4)

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Cattle	Hind leg	50	0(0)	6(17.6)	0(0)	4(13.3)	0(0)	10(11.1)
	Rectal	50	1(9.09)	5(14.7)	3(21.4)	5(16.6)	0(0)	14(15.5)
	Brisket	20	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
	Flank	20	2(18.1)	0(0)	0(0)	0(0)	0(0)	2(2.2)
	Hind leg	20	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
	Rectal	20	0(0)	0(0)	0(0)	0(0)	1(100)	1(1.1)
	Total	600	11(12.2)	34(37.7)	14(15.5)	30(33.3)	1(1.1)	90

A total of 150 animal species, 60% (sheep), 36.6% (goat) and 3.3% (cattle) were found positive as Enterococci. There was significant difference in prevalence ($P < 0.010$) between the *Enterococcus* species and animal species.

2.2. Virulence of Enterococci

The distribution of virulence genes among *Enterococcus* species is presented in Table 2. The different species of *Enterococcus* showed variability in their virulence gene profiles. The hyaluronidase virulence factor *hyl*, enterococcal surface protein *esp*, and cytolysin activator gene *cylA* were absent in all 90 of the *Enterococcus isolates.* Among the *E. faecalis* isolates, 6 (54.5%) isolates were tested positive for the *efaA gene. The* aggregation substance gene, *asaI*, was tested positive for 2 (18.8%) *E. faecalis* isolates. The collagen-binding protein gene, *ace*, and the gelatinase gene, *gelE*, were possessed by 1 (9.9%) and 1 (9.9%) *E. faecalis* isolates, respectively. Two, two, and one of five *E. hirae* isolates were tested positive for *gelE*, *efaA*, and *asaI* genes, respectively, whereas none of them possessed the *ace*, *esp*, *cylA*, or *hyl* genes. For *E. faecium* and *E. casseliflavus* isolates, 3/14 (21.4%) and 1/30 (3.3%) harbored *efaA* and *ace* genes, respectively.

Table 2. Distribution of virulence genes profiles among Enterococci.

Virulence genotypes	Number (%) of Enterococcus virulence factor genotypes					
	E. faecalis n=11	*E. hirae* n=34	*E. faecium* n=14	*E.casseliflavus* n=30	*E.gallinarum* n=1	Total (n = 90)
	n(%)	n(%)	n(%)	n(%)	n(%)	n(%)
ace	1(9.09)	0(0)	0(0)	1(3.3)	0(0)	2(2.2)
gelE	1(9.09)	2(18.8)	0(0)	0(0)	0(0)	3(3.3)
efaA	6(54.5)	2(18.8)	3(21.4)	0(0)	0(0)	11(12.2)
esp	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
asaI	2(18.8)	1(2.9)	0(0)	0(0)	0(0)	3(3.3)
cylA	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
hyl	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)

2.3. Antibiotic resistance

The resistance patterns of *Enterococci* towards the tested antimicrobial agents are presented in Table 3. Resistance to QD was the most common (28.8%), followed by TE (21.1%), AM (18%), and RA (15.5%). High rates of resistance to QD were found in *E. hirae* (21.1%) and *E. faecium* (7.7%). Tetracycline resistance was common in *E. faecalis* (6.6%), *E. casseliflavus* (6.6%), *E. hirae* (4.4%), and *E. faecium* (3.3%). Ampicillin and rifampin resistance were frequent in *E. faecium* (8.8%, 4.4%). Resistance to VA (7.7%), CIP (6.6%), F (4.4%), FF (4.4%), C (4.4%), P (2.2%), LEV (1.1%), and TIG (1.1%) was relatively low. Few isolates displayed HSLR (5.5%), which were in *E. faecalis, E. faecium,* and *E. casseliflavus*. except for *E. gallinarum*, 90 isolates of 4 species were found resistant to at least one antibiotic (64.4%, 58/90), with 17 isolates (18.8%, 17/90) of 90 *Enterococcus* species displaying multidrug resistance (Table 4).

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Table 3. Antimicrobial resistance pattern of Enterococci.

Antibiotic Group	Antibiotics	*Enterococcus* Species					
		E. faecalis n=11	*E. hirae* n=34	*E. faecium* n=14	*E.casseliflavus* n=30	*E. gallinarum* n=1	Total n=90
		n(%)	n(%)	n(%)	n(%)	n(%)	n(%)
Penicillins	AM	-	5 (5.5)	8(8.8)	4(4.4)	1(1.1)	18(20)
Penicillins	P	-	-	2(2.2)	-	-	2(2.2)
Lipoglycopeptides	TEC	-	-	-	-	-	-
Macrolides	E	*	3(3.3)	*	*	*	3(3.3)
Tetracyclines	TE	6(6.6)	4(4.4)	3(3.3)	6(6.6)	-	19(21.1)
Fluoroquinolones	CIP	1(1.1)	-	5(5.5)	-	-	6(6.6)
Fluoroquinolones	LEV	1(1.1)	-	-	-	-	1(1.1)
Nitrofurans	F	-	4(4.4)	-	-	-	4(4.4)
Ansamycins	RA	2(2.2)	4(4.4)	4(4.4)	3(3.3)	1(1.1)	14(15.5)
Fosfomycins	FF	-	-	2(2.2)	2(2.2)	-	4(4.4)
Phenicols	C	2(2.2)	-	1(1.1)	1(1.1)	-	4(4.4)
Streptogramins	QD	*	19(21.1)	7(7.7)	*	*	26(28.8)
Oxazolidinones	LNZ	-	-	-	-	-	-
Tetracyclines	TIG	-	-	-	1(1.1)	-	1(1.1)
Carbapenems	IPM	-	-	-	-	-	-
Glycopeptides	VA	3(3.3)	3(3.3)	1(1.1)	-	-	7(7.7)
Aminoglycosides	HSLR	1(1.1)	-	2(2.2)	2(2.2)	-	5(5.5)
Aminoglycosides	HGLR	-	-	-	-	-	-
MDR		4(36.3)	4(11.7)	7(30)	2(6.6)		17(18.8)

*; Intrinsic resistance; ampicillin, AM; penicillin, P; vancomycin, VA; teicoplanin, TEC; erythromycin, E; tetracycline, TE; ciprofloxacin, CIP; levofloxacin, LEV; nitrofurantoin, F; rifampin, RA; fosfomycin, FF; chloramphenicol, C; quinupristin-dalfopristin, QD; linezolid, LNZ; tigecycline, TIG; imipenem, IPM; high-level streptomycin-resistant, HLSR; high-level gentamicin-resistant, HLGR; vancomycin, VA; MDR, multidrug resistance.

Table 4. Characteristics of multidrug-resistant 17 Enterococci.

Strain	Animal species	Carcass part	Antibiotic resistance		Virulence factor
			Phenotype	Genotype	
EFM-4	Sheep	Hind leg	CIP, RA, QD		
EFM-7	Sheep	Hind leg	RA,P,AM		
EC-39	Goat	Rectal	HLSR, TE, AM		
EFM-45	Sheep	Rectal	CIP, RA, TE		
EH-48	Sheep	Rectal	E, AM, QD		
EH-49	Sheep	Brisket	RA, AM, QD	*ermB*	
EFM-57	Sheep	Rectal	HLSR, FF, AM,		
EH-66	Goat	Hind leg	RA, E, QD		
EFS-76	Sheep	Hind leg	C, CIP, LEV, RA, TE, AM	*aac(6')Ie-aph(2'')-la, Isa, efrA, tetM*	*efaA*
EFM-88	Goat	Flank	VA, AM, QD	*VanC1*	
EFS-97	Goat	Rectal	VA, C, HLSR, TE	*Isa, efrA, emeA, tetM*	*efaA*
EC-98	Sheep	Brisket	C, HLSR, AM, TE		
EFM-99	Goat	Rectal	C, HLSR, TE, FF, QD	*Isa, efrA, tetM*	
EFS-106	Cattle	Brisket	VA, TE, AM	*Isa, tetM*	
EFS-108	Sheep	Flank	VA, RA, TE, AM, QD	*Isa, tetM*	
EFM-113	Sheep	Hind leg	CIP, RA, TE, P, AM		

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EH-116	Goat	Hind leg	F, E, AM

EFM, *Enterococcus faecium*; EH, *Enterococcus hirae*; EFS, *Enterococcus faecalis*; EC, *Enterococcus casseliflavus*; ampicillin, AM; penicillin, P; erythromycin, E; tetracycline, TE; ciprofloxacin, CIP; levofloxacin, LEV; nitrofurantoin, F; rifampin, RA; fosfomycin, FF; chloramphenicol, C; quinupristin-dalfopristin, QD; high-level streptomycin-resistant, HLSR.

2.4. Genotyping of antibiotic resistance

The distribution of antibiotic-resistant elements amongst *Enterococcus* species is presented in Table 5 and Figure 1. Of the 3 isolates showing resistance to E, 1 (1.1%) carried the *ermA* gene, and 2 (2.2%) carried *aac(6′)Ie-aph(2″)-la*. The genes *efr*(A), *emeA*, and *lsa*, conferring efflux pump, were observed in 31 *Enterococcus* isolates. Glycopeptide genes like *VanC1* and *VanC2* were present in 1.1% and 1.1% of *E. faecium* and *E. hirae* isolates in goat and sheep carcasses, respectively.

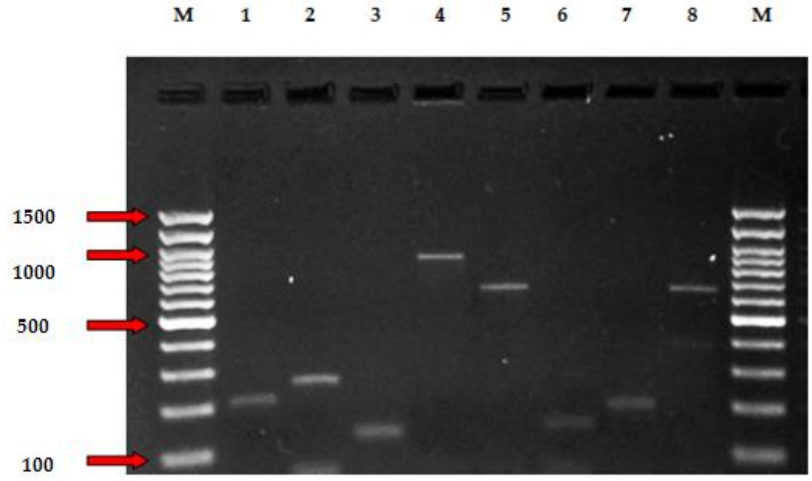

Figure 1. PCR assay revealing detection of genes for antibiotic resistance (*lsa*, *efr*(A), *emeA*, *vanC1*, *vanC2*, *ermA*, *ermB*, *tetM*) of Enterococcus isolates. lane M, DNA molecular weight marker (100 bp); Lane 1, *Enterococcus hirae Isa* 232 bp; lane 2, *Enterococcus faecalis efr(A)* 258 bp; lane 3, *Enterococcus casseliflavus emeA* 123 bp; 4, *Enterococcus faecium VanC1* 902 bp; lane 5, *Enterococcus hirae VanC2* 663 bp; lane 6, *Enterococcus hirae ermA* 200 bp; lane 7, *Enterococcus hirae ermB* 139 bp, lane 7; *Enterococcus faecalis tetM* 657 bp; lane M, DNA molecular weight marker (100 bp).

Table 5. The presence of antibiotic resistance genes profiles among Enterococci.

Antibiotic resistance genes	***Enterococcus* Species**					
	E. faecalis n=11	*E. hirae* n=34	*E. faecium* n=14	*E.casseliflavus* n=30	*E. gallinarum* n=1	Total (n = 90)
	n(%)	n(%)	n(%)	n(%)	n(%)	n(%)
ermA	*	1(1.1)	*	*	*	1(1.1)
ermB	*	2(2.2)	*	*	*	2(2.2)
mef	*	-	*	*	*	-
tet(L)	-	-	-	-	-	-
tet(M)	5(5.5)	-	-	1(1.1)	-	6(6.6)
tet(O)	-	-	-	-	-	-
cfr	-	-	-	-	-	-
fexA	-	-	-	-	-	-
optrA	-	-	-	-	-	-
aac(6′)Ie-aph(2″)-la	1(1.1)	-	-	-	-	1(1.1)
aph(2″)-Ib	-	-	-	-	-	-
aph(2″)-Ic	-	-	-	-	-	-
aph(2″)-Id	-	-	-	-	-	-

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ant(3'')-Ia	-	-	-	-	-	-
aph(6)-Ia	-	-	-	-	-	-
vanA	-	-	-	*	*	-
vanB	-	-	-	*	*	-
vanC1	-	-	1(1.1)	*	*	1(1.1)
vanC2	-	1(1.1)	-	*	*	1(1.1)
efr(A)	5(5.5)	2(2.2)	1(1.1)	1(1.1)	-	9(10)
lsa	9(10)	2(2.2)	1(1.1)	1(1.1)	-	13(14.4)
eme(A)	6(6.6)	1(1.1)	-	2(2.2)	-	9(10)

*; Intrinsic resistance, n; number.

3. Discussion

Enterococci, being a component of the normal microorganisms found in the gastrointestinal tract of animals, can be present in meat during the slaughtering process. The prevalent species include *E. hirae, E. faecium*, *E. faecalis*, *E. casseliflavus*, *E. mundtii*, *E. durans*, and *E. gilvus* [29]. Other people, the environment, and foods contaminated with livestock intestinal microflora are just a few of the ways that enterococci can spread to humans. These are only a few of these potential vectors [30]. The objective of this study was to determine the frequency of *Enterococcus* species, analyze their patterns of antibiotic resistance, and identify the presence of resistance and virulence genes in the *Enterococcus* species collected from Van, Turkey. This research is particularly relevant due to the high consumption of meat by a significant portion of the local population. In this study, *E. hirae* was the predominantly isolated species, which accounted for 37.7% of total *Enterococcus* isolates, and the remaining 33.3%, 15.5%, 12.2%, and 1.1% of the isolates were identified as *E. casseliflavus*, *E. faecium*, *E. faecalis, and E. gallinarum*, respectively (Table 1). Wide variation (0–90.6%) in the prevalence of Enterococci in food-producing animals has been reported in different countries [1,4,5,12,16,31–33]. In the present study, the speciation of the isolates confirmed that *E. hirae* was the most prevalent species identified from sheep and goat carcass samples. Other studies reported the prevalence of *Enterococcus* spp. on cattle at a slaugterhouse with a recovery rate of *E. hirae*, ranging from 8 to 92% [13,34]. Enterococci, especially *E. faecalis* and *E. faecium*, are known to be nosocomial pathogens and have become a major clinical concern [34]. Although *E. faecalis* and *E. faecium* isolates identified in this study are low-level documented by Ramos et al. [1] and Holman et al. [32], both species were isolated from slaughtered animals (12.2% and 15.5%, respectively). *E. casselifalavus* and *E. gallinarum* were also isolated from sheep carcasses and cattle carcasses, supporting similar findings reported by Ramos et al. [1] and Smoglica et al. [5]. The data regarding the occurrence of enterococci in cattle, sheep, and goat carcasses exhibits a wide range of variation. Differences in the occurrence rates of enterococci in cattle, sheep, and goat carcasses may be attributed to variances in geographical regions, hygiene conditions, livestock management practices, agro-climatic factors, detection sample methods, animal breeds, and age. Potential factors influencing the variability in results encompass the level of quality of the farms supplying the animals and the health and sickness conditions of the killed animals.

Given that enterococci are naturally found in the intestinal tract of animals, it is frequent for meat to get contaminated during the slaughtering process. Various enterococcal virulence genes associated with the initiation or worsening of illness symptoms in humans have been documented. Screening for certain genetic features in enterococci is necessary to identify their potential for causing disease and to confirm their ability to be transmitted between animals and humans, which is a significant health concern. The genetic transmission mechanisms are closely interconnected with the virulence traits of particular enterococci [36–38]. The identification of virulence factors is crucial in assessing bacterial pathogenicity, as these factors enable microorganisms to invade and harm the host. In this study, virulence typing was conducted by targeting seven specific genes. Of all the 90 isolates, 11 (12.2%) showed the presence of *efaA* genes. The other three *gelE*, *asaI*, and *ace* genes were present in 3 (3.3%), 3 (3.3%), and 2 (2.2%) isolates, respectively (Table 2). The high prevalence (12.2%) of endocarditis antigen *efaA* in *E. faecalis*, *E. hirae,* and *E. faecium* was consistent with findings from previous reports, whereas the moderate presence of *ace*, *gelE*, and *asaI* was lower than that previously

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reported by Beukers et al. [10], Zhang et al. [39], and Mohanty et al. [40]. Other authors have observed different values. Klibi et al. [12] in Tunisia detected *gelE*, *especially* in 11.5% and 10% of *Enterococcus* isolates in fecal samples from animals, respectively. Smoglica et al. [5] in Italy, in which the *gelE*, *asaI*, *efaA*, *ace*, and *esp* genes were observed in 35.41%, 25%, 22.91%, 0.08%, and 0.04% of *Enterococcus* isolates, respectively. In another study, Song et al. [8] reported that *E. faecalis* isolates were positive for *gelE* (88%), *asa1* (44%), *cylA* (16%), and *esp* (4%) virulence factor genes. Diversity in *enterococci* virulence genes reported from other studies might be attributable to various sampling techniques, sample types, isolation processes, environmental conditions, or geographic regions.

Enterococci are environmental organisms that have the ability to adapt to and spread antimicrobial-resistant traits [41]. Antimicrobial-resistant enterococci in animals are thought to serve as a reservoir for transmitting resistance genes to enterococci in humans. This transmission can occur through various means, such as human ingestion of animal-derived food, direct contact between animals and humans, or environmental factors. The visible limited preference of enterococci for certain hosts does not exclude the potential spread of antimicrobial resistance from animals to humans by enterococci [2]. In this investigation, *Enterococcus* isolates exhibited resistance to one or more antimicrobial agents, with a prevalence of 64.4%. The antibiotic resistance of *Enterococcus* isolates was assessed for 18 antimicrobial agents using the disk diffusion method. Among the 90 *Enterococcus* isolates examined, the most prevalent observation was resistance to quinupristin-dalfopristin (28.8%), followed by resistance to tetracycline (21.1%), ampicillin (20%), and rifampin (15.5%). None of the isolates were resistant to teicoplanin, linezolid, imipenem, or high-level gentamicin. *The Enterococcus* isolates were least resistant to vancomycin (7.7%), ciprofloxacin (6.6%), high-level streptomycin-resistant (5.5%), chloramphenicol (4.4%), fosfomycin (4.4%), nitrofurantoin (4.4%), erythromycin (3.3%), penicillin (2.2%), levofloxacin (1.1%), and tigecycline (1.1%) (Table 3). In this study, the high prevalence of tetracycline resistance, which was detected in enterococci other than antibiotics that belong to the natural resistance group, may be linked to the use of tetracyclines in the treatment of cattle, sheep, and goats. Oxytetracycline (OTC) is a tetracycline antibiotic primarily used to treat infections caused by a broad range of bacteria. However, it is important to note that resistance to OTC is frequently observed among gram-negative bacilli of enteric origin and staphylococci [42,43]. One prevalent application is in the treatment of bovine respiratory disease (BRD) in cattle, which is caused by *Pasteurella multocida*, *Mannheimia haemolytica*, and *Histophilus somni* (formerly known as *Haemophilus somnus*). Doxycycline is the preferred treatment for *Rickettsiae* and *Ehrlichiae* in small animals, as opposed to oxytetracycline [42,44]. Resistance to tetracyline in *Enterococcus* isolates was consistent with current results, which revealed a high resistant rate [1,4,45], but a higher prevalence of tetracycline-resistant *E. faecalis* was obtained in the present study [46–48]. A study conducted in Tunisia between September 2011 and December 2011 showed that *E. hirae* species isolated from animal stools were resistant to tetracyclines, in accordance with this study [12]. The *E. faecium* isolates were more resistant to ampicillin antimicrobial agents (20%). This result was in accordance with other study in Saudi Arabia that revealed similar result [9]. Resistance to rifampin in *E. casseliflavus* was consistent with the current result, which revealed a low resistance rate [16]. Compared with other antimicrobial agents, the resistance rates to ciprofloxacin were relatively low in *E. faecium* and *E. faecalis* (1.1%, 2.2%, respectively). These findings produced parallel results to those of other studies conducted in the USA and Korea [8,32]. The prevalence of antimicrobial-resistant *Enterococcus* strains in slaughtering and production can be explained by the extensive utilization of antibiotics for growth promotion, disease prevention, and infection treatment.

Multidrug resistance in enterococci can arise from either intrinsic attributes of the species or from acquired resistance mechanisms. The resistance to aminoglycosides can be attributed to both intrinsic and acquired factors. Resistance to high concentrations of gentamicin and streptomycin is usually acquired through the transfer of resistant genes, while resistance to low concentrations of amikacin, tobramycin, and kanamycin is frequently caused by intrinsic causes [49,50]. In this study, we have observed about 5.5% of HSLR *Enterococcus* isolates, whereas other findings have reported higher rates, including Li et al. (74.4%) [51]. Ngbede et al. [45] noted that 53.1% of 167 resistant isolates exhibited the highest multidrug resistance to antibiotics. Research conducted on cattle in South

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Australia [16] revealed that 26.9% of the isolates were resistant to at least two different classes of antibiotics. The values recorded by these authors were higher than the values noted in this study (18.8%).

Enterococci can gain resistance to clinically relevant medications through chromosomal mutations and horizontal gene transfer. Enterococci are naturally resistant to a wide range of antibiotic classes [15]. Enterococci present a major challenge to illness treatment because of their limited susceptibility to antibiotics, which is caused by both intrinsic and acquired resistances. These resistances enable them to obtain supplementary resistances on mobile genetic elements, resulting in heightened interaction with other antibiotic-resistant microorganisms [52]. Overall, 22 target antibiotic-resistant and efflux pump genes were detected at a variable frequency in tested isolates of *Enterococcus*, where *tet(M)*, *efr(A)*, *Isa*, and *eme(A)* were commonly detected compared to the *ermA*, *ermB*, *vanC1*, *vanC2*, and *aac(6')Ie-aph(2")-la* genes which were detected with less frequency (Table 5). *E. faecalis* harbored a greater number of *tet(M)* gene (5 isolates) than the other *Enterococcus* isolates, in agreement with a previous study [9]. Holman et al. [32] reported that the *tet(M)* antimicrobial resistance-encoding gene was detected in 31.9% (15 isolates) of *E. faecalis* bacteria. They also found that the *tet(M)* gene was detected in 37.5% (3 isolates) of *E. faecium*. You et al. [48] completed a report that was similar. They showed that the distribution of *tet(M) and aac(6')Ie-aph(2")-la* resistance genes in *E. faecalis* strains isolated from poultry in China was 91.80% and 67.21%, respectively. In another study, a Tunisian survey [12] reported that *E. faecalis* isolated from food-producing animals carried *aac(6')Ie-aph(2")* antimicrobial resistance-encoding genes. Thirty-one of the 85 *E. casseliflavus* isolates harbored the *tet*M gene. This result is similar to that reported in *E. casseliflavus* isolates from swine farms [53]. Out of the three genes, *ermA*, *ermB*, and *mef*, *ermB* was the most frequently detected in this investigation. It was found in 5.8% of *E. hirae* isolates (n = 2/34), which is consistent with findings from other studies [1,10,12,36]. *VanC1* and *VanC2* were identified in *E. faecium* and *E. hirae* isolates, respectively. These results are consistent with a previous study that identified *VanC1*/*VanC2* resistance genes in *E. faecium* isolates from aquaculture and slaughterhouse facilities [36]. Efflux pumps play a significant role in both natural and acquired resistance to antimicrobial medicines that are currently employed for the treatment of infectious illnesses [54]. Our finding that over 34.4% of enterococcal isolates carried efflux pump genes is consistent with previous reports from bovine feces, retail chicken meat, broiler chickens, and traditional fermented foods [10,28,55,56]. The changes in antibiotic use are likely to differ between nations due to variations in their usage patterns. Efflux pumps and the acquisition of genetic elements such as plasmids and transposons can result in increased tolerance or resistance to antimicrobials in enterococci.

4. Materials and Methods

4.1. Sample collection

Between November 2019 and December 2020, we obtained carcass samples from a total of 20 cattle, 80 sheep, and 50 goats from a slaughterhouse located in Van, a city in the eastern region of Turkey. The animals that were sampled were chosen in a randomized way. A visit was made to the chosen slaughterhouse in order to collect 600 surface samples from 150 animals and carcasses during the pre-chilling stage of the slaughtering process. A total of 600 samples from different regions of beef, sheep, and goat carcasses were collected using swabs. The sampling region was meticulously surveyed for one minute using cotton swabs that were swiped in both vertical and horizontal positions. Four 100 cm2 areas, measuring 10 cm2 × 10 cm2, were swabbed on each beef, sheep, and goat carcass. These areas were situated in the brisket, flank, hind leg, and rectal regions of the carcass. Carcass swabs were collected pre-chilling using sterile cotton swabs soaked in 10 ml of buffered peptone water, following the protocols set by the International Organization for Standardization (ISO) [20]. The sampler was pressed firmly and evenly as it was inserted vertically onto the peripheral surfaces, repeating this process approximately 10 times. Then, the sampler was turned and used to swipe horizontally and diagonally, each motion being repeated roughly 10 times. The samples were

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promptly delivered to the laboratory of Espiye Vocational School, Giresun University, within 24-48 hours of being collected, using refrigerated containers [21].

Figure 2. Carcasses before cooling.

4.2. Enterococus species isolation

Isolation was conducted as described previously. Swab samples were homogenized in a blender (Waring, New Hartford, Conn.) with 90 ml of buffered peptone water (BPW) (Lab M, Lancashire, UK). After inoculation at 37 °C for 24 h, 0.1 ml was streaked onto Slanetz and Bartley Agar (Lab M, Lancashire, UK) and incubated for 24 ± 2 h at 37 ± 1 °C under the same condition (Na et al., 2019). After incubating, we observed pink or dark red colonies with a narrow, whitish border. After the incubation period, five colonies that were believed to be *Enterococcus* spp. were selected from each petri dish and transferred to Tryptone Soya Agar (Lab M, Lancashire, UK) agar for purification. The agar plates were then incubated at a temperature of 37 ± 1 °C for 24 ± 2 hours. The suspected isolates were biochemically identified using Gram staining and catalase activity. All strains were kept in skim milk powder stocks at −80 °C for further testing [22]. The *Enterococcus* species were identified through MALDI-TOF MS (BioMérieux Inc., Marcy l'Etoile, France) performed only on gram-positive and catalase-negative cocci [23].

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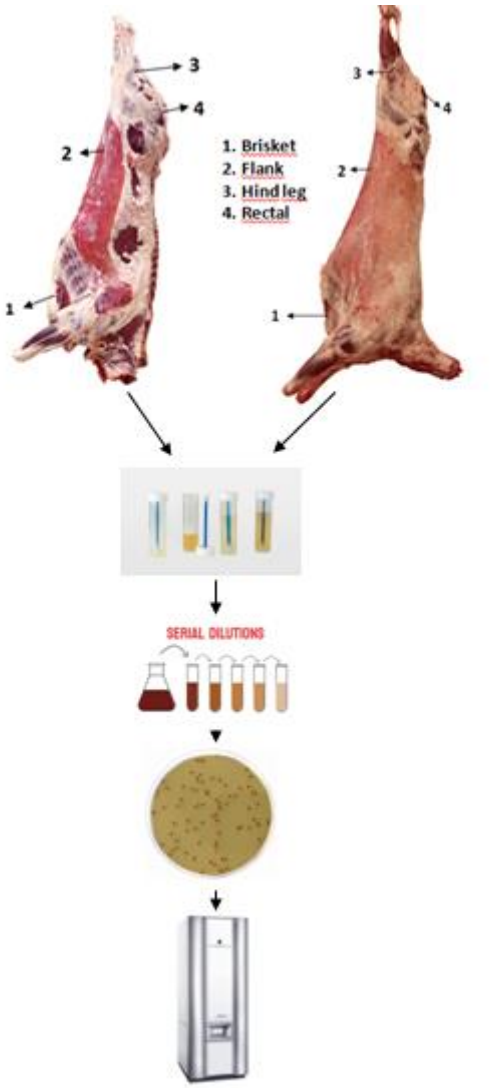

Figure 3. Isolation procedure of enterococci.

4.3. DNA isolation protocols

The QIAsymphony, a magnetic particle-based automated extraction system, was used to extract genomic DNA. The extraction was carried out using the QIAamp DNA micro kit (Qiagen, Hilden, Germany) following the instructions provided by the manufacturer. The isolated DNA was utilized as a template for PCR using the specified methods.

4.4. Screening for confirmation and virulence genes

Using PCR with the primers listed in Table 1, all *Enterococcus* isolates were screened for the confirmation genes and presence of virulence genes. These were 16S rDNA, *E. faecalis*, *E. faecium*, *E. hirae*, *E. casseliflavus*, *E. gallinarum* identification genes [24] and virulence genes; *asa1* (aggregation substance), *ace* (collagen-binding protein), *cylA* (cytolysin activator), *efaA* (endocarditis antigen), *esp* (enterococcal surface protein), *gelE* (gelatinase), *hyl* (hyaluronidase) [25]. The methods by Billström et al. (25] were modified and used for genotyping the *Enterococcus* isolates.

Table 6. Oligonucleotide primers for identification and virulence factors.

Target gene	Primer sequence (5'-3')	Fragment size (pb)
Enterococcus spp. *(16S rRNA)*	F: AGCGCAGGCGGTTTCTTAA R: CTCGTTGTACTTCCCATTGT	941
Enterococcus faecalis	F: ATCAAGTACAGTTAGTCTTTATTAG	658

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	R:ACGATTCAAAGCTAACTGAATCAGT	
Enterococcus faecium	F: TTGAGGCAGACCAGATTGACG R: GCTGCTAAAGCTGCGCTT	822
Enterococcus gallinarum	F: GGTATCAAGGAAACCTC R: CTTCCGCCATCATAGCT	484
Enterococcus casseliflavus	F: CGGGGAAGATGGCAGTAT R: CGCAGGGACGGTGATTTT	521
Enterococcus hirae	F: GCATATTTATCCAGCACTAG R: CTCTGGATCAAGTCCATAAGTGG	639
asa1	F: CACGCTATTACGAACTATGA R: TAAGAAAGAACATCACCACGA	375
ace	F: GGAATGACCGAGAACGATGGC R: GCTTGATGTTGGCCTGCTTCCG	616
cylA	F: ACTCGGGGATTGATAGGC R: GCTGCTAAAGCTGCGCTT	688
efaA	F: CGTGAGAAAGAAATGGAGGA R: CTACTAACACGTCACGAATG	499
esp	F: AGATTTCATCTTTGATTCTTG R: AATTGATTCTTTAGCATCTGG	510
gelE	F: TATGACAATGCTTTTTGGGAT R: AGATGCACCCGAAATAATATA	213
hyl	F: ACAGAAGAGCTGCAGGAAATG R: GACTGACGTCCAAGTTTCCAA	276

2.5. Antimicrobial susceptibility testing

The susceptibility of *Enterococcus* isolates to antibiotics was assessed using the disc diffusion method, following the protocols outlined by the Clinical and Laboratory Standards Institute (CLSI) [26]. To determine antibiotic resistance in the isolates, 10 µg ampicillin (AM), 5 µg ciprofloxacin (CIP), 30 µg chloramphenicol (C), 15 µg erythromycin (E), 200 µg fosfomycin (FF), 300 µg high-level streptomycin-resistant (HLSR), 120 µg high-level gentamicin-resistant (HLGR), 10 µg imipenem (IPM), 5 µg levofloxacin (LEV), 30 µg linezolid (LNZ), 300 µg nitrofurantoin (F), 10 units penicillin (P), 15 µg quinupristin-dalfopristin (QD), 5 µg rifampin (RA), 30 µg vancomycin (VA), 30 µg teicoplanin (TEC), 30 µg tetracycline (TE), 15 µg tigecycline (TIG) and 5 µg vancomycin (VA) antibiotic discs were used (all purchased from Liofilchem, Roseto degli Abruzzi, Italy). After incubation, the resulting diameters of the inhibition zones that formed around the discs of AM, P, VA-30 µg, TEC, E, TE, CIP, LEV, F, RA, FF, C, QD, LNZ, HLSR, and HLGR were classified as susceptible, intermediate, or resistant according to the diameters and breakpoints available in CLSI documents [26]. For the remaining antimicrobial agents (TIG, VA-5 µg, and IPM), the critical values were evaluated according to the zone table described by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) document [27]. For quality control purposes, *S. aureus* ATCC 25923 and *Enterococcus faecalis* ATCC 29212 were utilized as control strains.

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Figure 4. High-level streptomycin-resistant *Enterococcus casseliflavus* isolate.

2.6. PCR detection of genes for antimicrobial resistance

In the present study, various PCR assays were used for the detection of antibiotic resistance genes (AGRs) of *Enterococcus* isolates. All isolates were tested for the presence of aminoglycoside modifying enzyme (AME) genes [*aac*(6′)-*Ie*–*aph*(2′′)- *Ia*, *aph*(2′′)-*Ib*, *aph*(2′′)-*Ic*, *aph*(2′′)-*Id*, *ant*(3′′)-*Ia*, and *ant*(6)-*Ia*], phenicols resistance genes (*cfr*, *fexA*, and *optrA*), tetracyclines resistance genes [*tet*(L), *tet*(M), and *tet*(O)], macrolides resistance genes [*ermA*, *ermB* and *mef*], and efflux pump genes [*efr*(A), *emeA*, and *lsa*] by PCR using specific primers as described by a previous study [28], with some modifications (Table 2).

Table 7. Oligonucleotide primers for antimicrobial resistance genes.

Antimicrobial agent	Target gene	Primer sequence (5'-3')	Fragment size (pb)
Macrolides	*ermA*	F: TAACATCAGTACGGATATTG R: AGTCTACACTTGGCTTAGG	200
Macrolides	*ermB*	F: CCGAACACTAGGGTTGCTC R: ATCTGGAACATCTGTGGTATG	139
Macrolides	*mef*	F: AGTATCATTAATCACTAGTGC R: TTCTTCTGGTACTAAAAGTGG	348
Tetracyclines	*tet(L)*	F: ATAAATTGTTTCGGGTCGGTAAT R: AACCAGCCAACTAATGACAATGAT	1077
Tetracyclines	*tet(M)*	F: GTTAAATAGTGTTCTTGGAG R: CTAAGATATGGCTCTAACAA	657
Tetracyclines	*tet(O)*	F: GATGGCATACAGGCACAGAC R: CAATATCACCAGAGCAGGCT	614
Phenicols	*cfr*	F: TGAAGTATAAAGCAGGTTGGGAGTCA R: ACCATATAATTGACCACAAGCAGC	746
Phenicols	*fexA*	F: GTACTTGTAGGTGCAATTACGGCTGA R: CGCATCTGAGTAGGACATAGCGTC	1272
Phenicols	*optrA*	F: AGGTGGTCAGCGAACTAA R: ATCAACTGTTCCCATTCA	1379
Efflux pump	*eme(A)*	F: AGCCCAAGCGAAAAGCGGTTT R: CCATCGCTTTCGGACGTTCA	123
Efflux pump	*efr(A)*	F: GTCTGTTTCGTTTAATGGCAGCAGCC R: CGAATAGCTGGTTCATGTCTAAGGC	258
Efflux pump	*lsa*	F: GTGACTTCTTTTGAACAGTGGGA R: TTCAGCCACTTGTTGTCTGCC	232
Aminoglycoside modifying enzyme	*aac(6′)Ie-aph(2″)-la*	F: CAGAGCCTTGGGAAGATGAAG R: CCTCGTGTAATTCATGTTCTGGC	348
Aminoglycoside modifying enzyme	*aph(2″)-Ib*	F: CTTGGACGCTGAGATATATGAGCAC R: GTTTGTAGCAATTCAGAAACACCCTT	867
Aminoglycoside modifying enzyme	*aph(2″)-Ic*	F: CCACAATGATAATGACTCAGTTCCC R: CCACAGCTTCCGATAGCAAGAG	641
Aminoglycoside modifying enzyme	*aph(2″)-Id*	F: GTGGTTTTTACAGGAATGCCATC R: CCCTCTTCATACCAATCCATATAACC	284
Aminoglycoside modifying enzyme	*ant(3″)-Ia*	F: TGATTTGCTGGTTACGGTGAC R: CGCTATGTTCTCTTGCTTTTG	284
Aminoglycoside modifying enzyme	*aph(6)-Ia*	F: ACTGGCTTAATCAATTTGGG R: GCCTTTCCGCCACCTCACCG	596
Glycopeptides	*vanA*	F: ATTGCTATTCAGCTGTACTC	559

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Glycopeptıdes	*vanB*	R: GGCTCGACTTCCTGATGAAT F: AACGGCGTATGGAAGCTATG R: CCATCATATTGTCCTGCTGC	467
Glycopeptıdes	*vanC1*	F: GGCATCGCACCAACAATGGA R: TCCTCTGCCAGTGCAATCAA	902
Glycopeptıdes	*vanC2*	F: TTCAGCAACTAGCGCAATCG R: TCACAAGCACCGACAGTCAA	663

2.7. Statistical analysis

Descriptive statistics for the categorical variables in this study, which was conducted for the purpose of animal species, animal carcass sites, and *Enterococcus* species expressed as a number (n) and a percentage (%). "Chi-square" and "Fisher's exact" tests were calculated to determine the relationships between the factors "animal species, carcass sites, and *Enterococcus* species." In the calculations, the statistical significance level was taken as $p<0.05$, and the SPSS (IBM SPSS for Windows, ver. 26) statistical package program was used for analyses.

5. Conclusions

This study revealed information about the similarity of antibiotic resistance and virulence-related genes of enterococci isolated from animals to human enterococci. The presence of the same mobile DNA elements in these strains of both human and animal origin may indicate horizontal transfer of this resistance gene. The isolation of virulence potential and multidrug-resistant enterococci isolates from slaughtered cattle, sheep, and goat carcasses emphasized the importance of slaughter hygiene in the transmission of pathogenic enterococci. The presence of enterococci in different parts of carcasses and during the pre-chilling stage poses a risk of cross-contamination in the examined facility. Carcasses can be contaminated with fecal bacteria, the majority of which results from contamination during the slaughter process, such as damage to intestinal tissue during evisceration and fecal leakage, which can increase cross-contamination of carcasses. According to the data determined in this study, enterococci may pose a potential risk to public health, considering their virulence potential and antibiotic resistance (mainly against quinupristin-dalfopristin, tetracycline, and ampicillin). Therefore, considering the slaughterhouse in Van Province included in this study, larger studies from different geographical regions are needed to fully understand the genetic diversity of enterococci in farm animals.

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References

1. Ramos, S.; Igrejas, G.; Capelo-Martinez, J. L.; Poeta, P. Antibiotic resistance and mechanisms implicated in fecal enterococci recovered from pigs, cattle and sheep in a Portuguese slaughterhouse. *Annals of microbiology* **2012**, *62*(4), 1485-1494.
2. Angulo, F. J.; Heuer, O. E.; Hammerum, A. M.; Collignon, P.; Wegener, H. C. Human health hazard from antimicrobial-resistant enterococci in animals and food. *Clinical Infectious Diseases* **2006**, *43*(7), 911-916.
3. Na, S. H.; Moon, D. C.; Choi, M. J.; Oh, S. J.; Jung, D. Y.; Kang, H. Y.; Hyun, S. J.; Lim, S. K. Detection of oxazolidinone and phenicol resistant enterococcal isolates from duck feces and carcasses. *International journal of food microbiology* **2019**, *293*, 53-59.

4. Kim, M. H.; Moon, D. C.; Kim, S. J.; Mechesso, A. F.; Song, H. J.; Kang, H. Y.; Choi, J. Y.; Yoon, S. S.; Lim, S. K. Nationwide surveillance on antimicrobial resistance profiles of *Enterococcus faecium* and *Enterococcus faecalis* isolated from healthy food animals in South Korea, 2010 to 2019. *Microorganisms* **2021**, *9(5)*, 925.
5. Smoglica, C.; Vergara, A.; Angelucci, S.; Festino, A. R.; Antonucci, A.; Marsilio, F.; Di Francesco, C. E. Evidence of linezolid resistance and virulence factors in *Enterococcus* spp. isolates from wild and domestic ruminants, Italy. *Antibiotics* **2022**, *11*(2), 223.
6. Desire, O. E.; Larson, B.; Richard, O.; Rolande, M. M.; Serge, K. B. Investigating antibiotic resistance in enterococci in Gabonese livestock. *Veterinary World* **2022**, *15*(3), 714.
7. Strateva, T.; Atanasova, D.; Savov, E.; Petrova, G.; Mitov, I. Incidence of virulence determinants in clinical *Enterococcus faecalis* and *Enterococcus faecium* isolates collected in Bulgaria. *Brazilian Journal of Infectious Diseases* **2016**, *20*, 127-133.
8. Song, H.; Bae, Y.; Jeon, E.; Kwon, Y.; Joh, S. Multiplex PCR analysis of virulence genes and their influence on antibiotic resistance in *Enterococcus* spp. isolated from broiler chicken. *Journal of veterinary science* **2019**, *20*(3).
9. Alzahrani, O. M.; Fayez, M.; Alswat, A. S.; Alkafafy, M.; Mahmoud, S. F.; Al-Marri, T.; Almuslem, A.; Ashfaq, H.; Yusuf, S. Antimicrobial resistance, biofilm formation, and virulence genes in *Enterococcus* species from small backyard chicken flocks. *Antibiotics* **2022**, *11*(3), 380.
10. Beukers, A. G.; Zaheer, R.; Goji, N.; Amoako, K. K.; Chaves, A. V.; Ward, M. P.; McAllister, T. A. Comparative genomics of *Enterococcus* spp. isolated from bovine feces. *BMC microbiology* **2017**, *17*(1), 1-18.
11. Ribeiro, J.; Silva, V.; Monteiro, A.; Vieira-Pinto, M.; Igrejas, G.; Reis, F. S.; Barros, L.; Poeta, P. Antibiotic Resistance among Gastrointestinal Bacteria in Broilers: A Review Focused on *Enterococcus* spp. and *Escherichia coli*. *Animals* **2023**, *13*(8), 1362.
12. Klibi, N.; Aouini, R.; Borgo, F.; Ben Said, L.; Ferrario, C.; Dziri, R., Boudabous, A.; Torres, C.; Ben Slama, K. Antibiotic resistance and virulence of faecal enterococci isolated from food-producing animals in Tunisia. *Annals of Microbiology* **2015**, *65*(2), 695-702.
13. Zaheer, R.; Cook, S. R.; Barbieri, R.; Goji, N.; Cameron, A.; Petkau, A.; Polo, R. O.; Thymensen, L.; Stamm, C.; Song, J.; Hannon, S.; Jones, T.; Church, D.; Booker, C. W.; Amoako, K.; Domselaar, G., V.; Read, R. R.; McAllister, T. A. Surveillance of *Enterococcus* spp. reveals distinct species and antimicrobial resistance diversity across a One-Health continuum. *Sci Reports* **2020**, *10 (1)*, 1–16.
14. Dafale, N. A.; Srivastava, S.; Purohit, H. J. Zoonosis: an emerging link to antibiotic resistance under "one health approach". *Indian journal of microbiology* **2020**, *60*, 139-152.
15. Bortolaia, V.; Guardabassi, L. Zoonotic Transmission of Antimicrobial-Resistant Enterococci: A Threat to Public Health or an Overemphasized Risk?. In *Zoonoses: Infections Affecting Humans and Animals*, Sing, A. Ed.; Cham: Springer International Publishing, Germany, 2023; pp. 1-33.
16. Wambui, J.; Tasara, T.; Njage, P. M. K.; Stephan, R. Species distribution and antimicrobial profiles of *Enterococcus* spp. isolates from Kenyan small and medium enterprise slaughterhouses. *Journal of food protection* **2018**, *81*(9), 1445-1449.
17. European Food Safety Authority Panel on Biological Hazards-BIOHAZ. Scientific Opinion on the public health hazards to be covered by inspection of meat (bovine animals). *EFSA Journal* **2013**, *11*(6), 3266.
18. European Food Safety Authority Panel on Biological Hazards-BIOHAZ. Scientific Opinion on the public health hazards to be covered by inspection of meat from sheep and goats. *EFSA Journal* **2013**, *11*(6), 3265.
19. Wardhana, D. K. Risk factors for bacterial contamination of bovine meat during slaughter in ten Indonesian abattoirs. *Veterinary medicine international* **2019**.
20. *Microbiology of the food chain – Carcass sampling for microbiological analysis, ISO 17604*; International Organization for Standardization : Geneva, Switzerland, 2015.
21. Cebeci, T. *Listeria monocytogenes* in Ruminants at an Abattoir: Prevalence, Virulence Characteristics, Serotypes and Antibiotic Resistance in Eastern Türkiye. *Israel Journal of Veterinary Medicine* **2022**, *77*, 4.
22. Pesavento, G.; Calonico, C.; Ducci, B.; Magnanini, A.; Nostro, A. L. Prevalence and antibiotic resistance of *Enterococcus* spp. isolated from retail cheese, ready-to-eat salads, ham, and raw meat. *Food microbiology* **2014**, *41*, 1-7.
23. Quintela-Baluja, M.; Böhme, K.; Fernández-No, I. C.; Morandi, S.; Alnakip, M. E.; Caamaño-Antelo, S.; Barros-Velázquez, J.; Calo-Mata, P. Characterization of different food-isolated *Enterococcus* strains by MALDI-TOF mass fingerprinting. *Electrophoresis* **2013**, *34*(15), 2240-2250.

24. Chotinantakul, K.; Chansiw, N.; Okada, S. Antimicrobial resistance of *Enterococcus* spp. isolated from Thai fermented pork in Chiang Rai Province, Thailand. *Journal of Global Antimicrobial Resistance* **2018**, *12*, 143-148.
25. Billström, H.; Lund, B.; Sullivan, Å.; Nord, C. E. Virulence and antimicrobial resistance in clinical *Enterococcus faecium*. *International journal of antimicrobial agents* **2008**, *32*(5), 374-377.
26. *Performance standards for antimicrobial susceptibility testing, CLSI supplement M100*, 33th ed.; Clinical and Laboratory Standards Institute: Wayne, PA, USA, 2023.
27. *The European Committee on Antimicrobial Susceptibility Testing, EUCAST supplement 2023*, version 13; Breakpoint tables for interpretation of MICs and zone diameters, , The European Committee: Växjö, Sweden, 2023.
28. Kim, Y. B.; Seo, K. W.; Son, S. H.; Noh, E. B.; Lee, Y. J. Genetic characterization of high-level aminoglycoside-resistant *Enterococcus faecalis* and *Enterococcus faecium* isolated from retail chicken meat. *Poultry Science* **2019**, *98*(11), 5981-5988.
29. Ben Braiek, O.; Smaoui, S. Enterococci: between emerging pathogens and potential probiotics. *BioMed research international* 2019, *2019*, 5938210.
30. Efstratiou, A.; Lamagni, T.; Turner, C. E. Streptococci and Enterococci. In *Infectious Diseases*, 4th ed.; Cohen, J., Powderly, W. G., Opal, S. M.; Elsevier: China, 2017; Volume 2, pp. 1523-1536.
31. Holman, D. B.; Gzyl, K. E.; Zaheer, R.; Jones, T. H.; McAllister, T. A. Draft genome sequences of 43 *Enterococcus faecalis* and *Enterococcus faecium* isolates from a commercial beef processing plant and retail ground beef. *Microbiology Resource Announcements* 2019, *8*(42), 10-1128.
32. Holman, D. B.; Klima, C. L.; Gzyl, K. E.; Zaheer, R.; Service, C.; Jones, T. H.; McAllister, T. A. A Longitudinal Study of Antimicrobial Resistance in *Enterococcus* spp. Isolated from a Beef Processing Plant and Retail Ground Beef. *bioRxiv* **2021**, 2021-05.
33. Telli, N.; Telli, A. E.; Biçer, Y.; Turkal, G.; Uçar, G. Isolation and antimicrobial resistance of vancomycin resistant *Enterococcus* spp.(VRE) and methicillin-resistant *S. aureus* (MRSA) on beef and chicken meat, and workers hands from slaughterhouses and retail shops in Turkey. *Journal of the Hellenic Veterinary Medical Society* **2021**, *72*(4), 3345-3354.
34. Messele, Y. E.; Hasoon, M. F.; Trott, D. J.; Veltman, T.; McMeniman, J. P.; Kidd, S. P.; Low, W. Y.; Petrovski, K. R. Longitudinal Analysis of Antimicrobial Resistance among *Enterococcus* Species Isolated from Australian Beef Cattle Faeces at Feedlot Entry and Exit. *Animals* **2022**, *12*(19), 2690.
35. Guzman Prieto, A. M.; van Schaik, W.; Rogers, M. R.; Coque, T. M.; Baquero, F.; Corander, J.; Willems, R. J. Global emergence and dissemination of enterococci as nosocomial pathogens: attack of the clones?. *Frontiers in microbiology* **2016**, *7*, 788.
36. Igbinosa, E. O.; Beshiru, A. Antimicrobial resistance, virulence determinants, and biofilm formation of *Enterococcus* species from ready-to-eat seafood. *Frontiers in Microbiology* **2019**, *10*, 728.
37. Golob, M.; Pate, M.; Kušar, D.; Dermota, U.; Avberšek, J.; Papić, B.; Zdovc, I. Antimicrobial resistance and virulence genes in *Enterococcus faecium* and *Enterococcus faecalis* from humans and retail red meat. *BioMed research international* ***2019*.**
38. Fiore, E.; Van Tyne, D.; Gilmore, M. S. Pathogenicity of enterococci. *Microbiology spectrum* **2019**, *7*(4), 7-4.
39. Zhang, F., Jiang, M., Wan, C., Chen, X., Chen, X., Tao, X., Shah, N. P.; Wei, H. Screening probiotic strains for safety: Evaluation of virulence and antimicrobial susceptibility of enterococci from healthy Chinese infants. *Journal of dairy science* 2016, *99*(6), 4282-4290.
40. Mohanty, S.; Behera, B. Antibiogram Pattern and Virulence Trait Characterization of *Enterococcus* Species Clinical Isolates in Eastern India: A Recent Analysis. *Journal of Laboratory Physicians* **2022**, *14*(03), 237-246.
41. Ahmed, M. O.; Baptiste, K. E. Vancomycin-resistant enterococci: a review of antimicrobial resistance mechanisms and perspectives of human and animal health. *Microbial Drug Resistance* **2018**, *24*(5), 590-606.
42. Papich, M. G. Oxytetracycline. In *Saunders Handbook of Veterinary Drug Small and Large Animal*, 4th ed.; Papich, M. G.; Sounders: USA, 2016; pp. 595-598.
43. Ayeni, F. A.; Odumosu, B. T.; Oluseyi, A. E.; Ruppitsch, W. Identification and prevalence of tetracycline resistance in enterococci isolated from poultry in Ilishan, Ogun State, Nigeria. *Journal of pharmacy & bioallied sciences* 2016, *8(1)*, 69.
44. Smith, M. V. Therapeutics. In *Textbook of Rabbit Medicine*, 3rd ed.; Smith, M. V.; Elsevier: Poland, 2023; pp. 100-137.

45. Ngbede, E. O.; Raji, M. A.; Kwanashie, C. N.; Kwaga, J. K. P. Antimicrobial resistance and virulence profile of enterococci isolated from poultry and cattle sources in Nigeria. *Tropical animal health and production* 2017, *49*, 451-458.
46. Barlow, R. S.; McMillan, K. E.; Duffy, L. L.; Fegan, N.; Jordan, D.; Mellor, G. E. Antimicrobial resistance status of *Enterococcus* from Australian cattle populations at slaughter. *PLoS One* **2017**, *12*(5), e0177728.
47. Xuan, H.; Yao, X.; Pan, R.; Gao, Y.; Wei, J.; Shao, D.; Liu, K.; Li, Z.; Qiu, Y.; Ma, Z.; Li, B.; Xia, L. Antimicrobial resistance in *Enterococcus faecium* and *Enterococcus faecalis* isolates of swine origin from eighteen provinces in China. *Journal of Veterinary Medical Science* **2021**, *83*(12), 1952-1958.
48. Yu, L.; Liu, Y.; Liu, M.; Li, Z.; Li, L.; Wang, F. Research Note: Molecular characterization of antimicrobial resistance and virulence gene analysis of *Enterococcus faecalis* in poultry in Tai'an, China. *Poultry Science* **2022**, *101*(5), 101763.
49. Hollenbeck, B. L.; Rice, L. B. Intrinsic and acquired resistance mechanisms in Enterococcus. *Virulence* **2012**, 3(5), 421–569.
50. Jian, Z., Zeng, L., Xu, T., Sun, S., Yan, S., Yang, L., Huang, Y.; Jia, J.; Dou, T. Antibiotic resistance genes in bacteria: Occurrence, spread, and control. *Journal of basic microbiology* **2021**, *61*(12), 1049-1070.
51. Li, W.; Li, J.; Wei, Q.; Hu, Q.; Lin, X.; Chen, M.; Ye, R.; Lv, H. Characterization of aminoglycoside resistance and virulence genes among *Enterococcus* spp. isolated from a hospital in China. *International journal of environmental research and public health* 2015, *12*(3), 3014-3025.
52. Kristich, C. J.; Rice, L. B.; Arias, C. A. Enterococcal infection—treatment and antibiotic resistance. In *Enterococci: from commensals to leading causes of drug resistant infection [Internet]*, Gilmore, M. S., Ed.; Massachusetts Eye and Ear Infirmary: Boston, USA, 2014; pp. 1-47.
53. Channaiah, L. H.; Subramanyam, B.; Zurek, L. Molecular characterization of antibiotic resistant and potentially virulent enterococci isolated from swine farms and feed mills. *Journal of Stored Products Research* 2018, *77*, 189-196.
54. Hernando-Amado, S.; Blanco, P.; Alcalde-Rico, M.; Corona, F.; Reales-Calderón, J. A.; Sánchez, M. B.; Martínez, J. L. Multidrug efflux pumps as main players in intrinsic and acquired resistance to antimicrobials. *Drug Resistance Updates* **2016**, *28*, 13-27.
55. Sanchez Valenzuela, A.; Lavilla Lerma, L.; Benomar, N.; Gálvez, A.; Perez Pulido, R.; Abriouel, H. Phenotypic and molecular antibiotic resistance profile of *Enterococcus faecalis* and *Enterococcus faecium* isolated from different traditional fermented foods. *Foodborne pathogens and disease* **2013**, *10*(2), 143-149.
56. Rehman, M. A.; Yin, X., Zaheer; R., Goji; N., Amoako; K. K., McAllister, T., Pritchard, J.; Diarra, M. S. Genotypes and phenotypes of Enterococci isolated from broiler chickens. *Frontiers in Sustainable Food Systems* **2018**, *2*, 83.