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The prevalence, vancomycin resistance and virulence gene profiles of *Enterococcus* species recovered from different foods of animal origin

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ABSTRACT

In this study, *Enterococcus faecium* was the most commonly found species with a level of 10.1%, followed by *Enterococcus durans* (19/246, 7.7%), *Enterococcus faecalis* (13/246, 5.2%), and *Enterococcus hirae* (9/246, 3.6%). When the virulence gene profile of isolates was evaluated, *gelE* was the predominant (25/66, 37.8%) virulence factor in isolates followed by *asa1* (22/66, 33.3%), *esp* (12/66, 18.1%), and *cylA* (4/66, 6.0%). None of the isolates harboured the *hyl* gene. In this study, all and/or the majority of the *Enterococcus* isolates tested were found to be resistant to ampicillin, rifampicin, vancomycin, and erythromycin. However, vancomycin resistance genes, such as *vanB* and *vanC1*, were not determined in any of the isolates by multiplex PCR. Only three isolates of *E. durans* recovered from Surk cheese were found to be carrying the *vanA* gene. The results demonstrate that antimicrobial-resistant and virulent strains of enterococci occur in food of animal origin in Turkey.

Key words: antibiotic resistance; cheese, *Enterococcus*; *tuf* gene; virulence

Introduction

Enterococci are Gram-positive, catalase-negative, thermotolerant, facultative anaerobic bacteria. The gastrointestinal tracts of humans and animals serve as a primary host of these bacteria. Moreover, enterococci are ubiquitous bacteria that are also found in soil, water, vegetables and animal foods. They have the ability to grow at a wide range of temperatures and pH values. Enterococci are relatively resistant to heat processing and may survive during classic milk pasteurization and cause spoilage (FRANZ et al., 1999; AARESTRUP et al., 2000; HAYES et al., 2003; BUSANI et al., 2004; SANTESTEVAN et al., 2015).

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Enterococci have been known as indicators of fecal contamination. On the other hand, fermented meat and dairy products usually contain enterococci at different levels. Since they are important for ripening and flavor development in fermented foods, their presence is not considered as an indicator of fecal contamination in such kinds of foods. Some *Enterococcus* species have probiotic properties and also produce bacteriocins. Again, some enterococci are therapeutically used in the treatment of gastroenteritis in humans and animals. On the other hand, enterococci are considered as important nosocomial pathogens, because they cause bacteraemia, endocarditis, urinary tract and neonatal infections (FRANZ et al., 1999; RADU et al., 2001; GOMES et al., 2008; FRAZZON et al., 2010).

Enterococci have increasing resistance to antibiotics used in clinical practice. As they cause nosocomial infections, an important concern is the occurrence of difficulties in the treatment of infections in humans. In enterococci, antimicrobial resistance is acquired by gene transfer systems or is found intrinsically. Vancomycin resistance has been described as transferable by conjugation. It has been suggested that excessive use of antibiotics in clinical practice has led to the emergence of vancomycin-resistant enterococci (VRE). VRE were first isolated in Europe and quickly spread to the USA (AARESTRUP et al., 2000; SCHOUTEN et al., 2000; HAYES et al., 2003; BUSANI et al., 2004; JUNG et al., 2007; FRAZZON et al., 2010).

The occurence of antimicrobial resistant enterococci has been identified in food animals. As a result, food of animal origin may be a source of resistant enterococci. Antibiotic resistance genes may be transferred to humans via foods (FRANZ et al., 1999; ROBREDO et al., 2000; RADU et al., 2001). Therefore, it is very important to detect the presence of resistant enterococci in foods. For better understanding of the pathogenesis of enterococci, both antibiotic resistance and virulence gene profiles should be considered together. In Turkey, information about the occurence of virulent and antimicrobial resistant enterococci in food of animal origin is limited. Therefore, the present study was conducted to determine the prevalence, antibiotic resistance and virulence gene profiles of *Enterococcus* species in different foods of animal origin.

Materials and methods

Sample collection. In this study, a total of 225 samples, including 40 ground beef, 40 frozen broiler wing meat, 30 Carra cheese, 30 Surk cheese, 30 Künefe cheese, and 55 cow's milk (bulk tank milk) samples, were obtained in the province of Hatay. The samples were brought to the laboratory under cold chain and analysed microbiologically on the same day.

Isolation and identification of enterococci. In this study, isolation of vancomycin-susceptible enterococci (VSE) and vancomycin-resistant enterococci (VRE) was performed using the classical culture method, as described below (OXOID, 2014).

For the isolation of VSE strains, 10 g and/or 10 mL of each sample were suspended in 90 mL of VRE broth (Oxoid, Basingstoke, Hampshire, England) supplemented with meropenem (Oxoid) and incubated at 37 °C for 18-22 h. Then, a loopful of growth was plated on VRE agar (Oxoid) and incubated at 37 °C for 24-48 h.

For isolation of the VRE strains, 10 g and/or 10 mL of each sample were added to 90 mL of VRE broth containing meropenem, and incubated at 37 °C for 18-22 h. After incubation, a loopful of growth was streaked onto VRE agar supplemented with vancomycin (Oxoid) and incubated at 37 °C for 24-48 h.

Typical colonies for enterococci (grey and/or pale brown colonies with black halos) from VRE agar were randomly collected and then, the following tests were applied to these colonies: Gram staining, catalase production, hemolytic activity, growth on Brain Heart Infusion agar (Oxoid) supplemented with 6.5% (w/v) NaCI. After identification of *Enterococcus* spp. and VRE by the classical culture method, isolates were stored at -20 °C until the PCR analysis.

PCR analysis. For PCR analysis, DNA extraction from the isolates was performed using a Bacterial DNA Extraction kit (Nucleic Acid Extraction Kit, Vivantis, Malaysia), following the manufacturer's instructions. After the extraction, all DNA's were stored at -20 °C until the PCR confirmation.

Confirmation of enterococci at genus level by PCR assay. All *Enterococcus* isolates were confirmed by PCR based on the detection of the genus specific *tuf* gene. In this study, Ent1 and Ent2 primers (Ella Biotech GmbH, Martinsried, Germany) described by KE et al. (1999) were used (Table 1). For the PCR assay, reaction mixture and amplification conditions were performed as by KASIMOGLU-DOGRU et al. (2010).

Confirmation of enterococci at species level by multiplex PCR assay. For confirmation of *Enterococcus faecalis, Enterococcus faecium, Enterococcus hirae,* and *Enterococcus durans*, primer pairs reported by CHENG et al. (1997), KARIYAMA et al. (2000), and JACKSON et al. (2004) were used. The sequences of these primers are shown in Table 1.

In the multiplex PCR analysis, two different reaction mixtures, described by KASIMOGLU-DOGRU et al. (2010), were carried out. DNA amplification conditions suggested by KARIYAMA et al. (2000) were used for detection of *E. faecalis* and *E. faecium,* while for *E. hirae* and *E. durans,* JACKSON et al. (2004) thermal cycling protocol was adopted.

Molecular analysis of virulence genes among the E. faecalis, E. faecium, E. hirae and E. durans isolates. Virulence genes (*asa1, gelE, cylA, esp* and *hyl*) were determined

by multiplex PCR in all *E. faecalis, E. faecium, E. hirae* and *E. durans* isolates. Specific primers described by VANKERCKHOVEN et al. (2004), COQUE et al. (1995), WILLEMS et al. (2001) were used for this purpose (Table 1). PCR reaction mixture and amplification conditions were carried out according to VANKERCKHOVEN et al. (2004) and BUYUKYORUK et al. (2014), as previously reported.

Table 1. Specific primers used for identificaton of *Enterococcus* isolates, detection of virulence and vancomycin resistance genes among the isolates in the present study

Genes	Sequence (5'-3')	Size (bp)	References
tuf	Ent1- TACTGACAAACCATTCATGATG Ent2- AACTTCGTCACCAACGCGAAC	112	Ke et al. (1999)
$ddl_{E. faecalis}$	ddlE1-ATCAAGTACAGTTAGTCTTTATTAG ddlE2-ACGATTCAAAGCTAACTGAATCAGT	941	Kariyama et al. (2000)
$ddl_{E. faecium}$	ddlF1- TTGAGGCAGACCAGATTGACG ddlF2- TATGACAGCGACTCCGATTCC	658	Cheng et al. (1997)
$ddl_{E. durans}$	DU1- CCTACTGATATTAAGACAGCG DU2- TAATCCTAAGATAGGTGTTTG	295	Jackson et al. (2004)
$ddl_{E. hirae}$	HI1- CTTTCTGATATGGATGCTGTC HI2- TAAATTCTTCCTTAAATGTTG	187	Jackson et al. (2004)
asa1	ASA11- GCACGCTATTACGAACTATGA ASA12- TAAGAAAGAACATCACCACGA	375	Vankerckhoven et al. (2004)
gelE	GEL11- TATGACAATGCTTTTTGGGAT GEL12- AGATGCACCCGAAATAATATA	213	Vankerckhoven et al. (2004)
cylA	CYT I- ACTCGGGGATTGATAGGC CYT IIb- GCTGCTAAAGCTGCGCTT	688	Coque et al. (1995)
esp	ESP 14F- AGATTTCATCTTTGATTCTTGG ESP 12R- AATTGATTCTTTAGCATCTGG	510	Willems et al. (2001)
hyl	HYL n1- ACAGAAGAGCTGCAGGAAATG HYL n2- GACTGACGTCCAAGTTTCCAA	276	Vankerckhoven et al. (2004)
vanA	A1- CATGAATAGAATAAAAGTTGCAATA A2- CCCCTTTAACGCTAATACGATCAA	1030	Evers et al. (1993)
vanB	B1- GTGACAAACCGGAGGCGAGGA B2- CCGCCATCCTCCTGCAAAAAA	433	Handwerger et al. (1992)
vanC1	C1- GGTATCAAGGAAACCTC C2- CTTCCGCCATCATAGCT	822	Dutka-Malen et al. (1995)

Determination of antimicrobial susceptibility. All *Enterococcus* isolates were tested for susceptibility to the following antibiotics, using the disk diffusion method: ampicillin (10 µg/disc), streptomycin (300 µg/disc), gentamicin (120 µg/disc), chloramphenicol (30 µg/disc), rifampicin (5 µg/disc), ciprofloxacin (5 µg/disc), erythromycin (15 µg/disc), and vancomycin (30 µg/disc). The results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2013).

PCR amplification of vancomycin resistance genes. Specific primer pairs were selected for vancomycin resistance genes (*vanA, vanB,* and *vanC1*) according to EVERS et al. (1993), HANDWERGER et al. (1992), and DUTKA-MALEN et al. (1995) (Table 1). PCR analysis was conducted according to KARIYAMA et al. (2000).

Results

In this study, a total of 225 samples were examined and 155 (68.8%) of them were found to be contaminated with *Enterococcus* spp. Enterococci were isolated from 52 (57.7%), 33 (60%), 33 (82.5%), and 37 (92.5%) cheese, raw milk, broiler meat, and ground beef samples, respectively. A total of 246 isolates were confirmed as *Enterococcus* spp. by PCR, based on the detection of the *tuf* gene (Fig. 1).

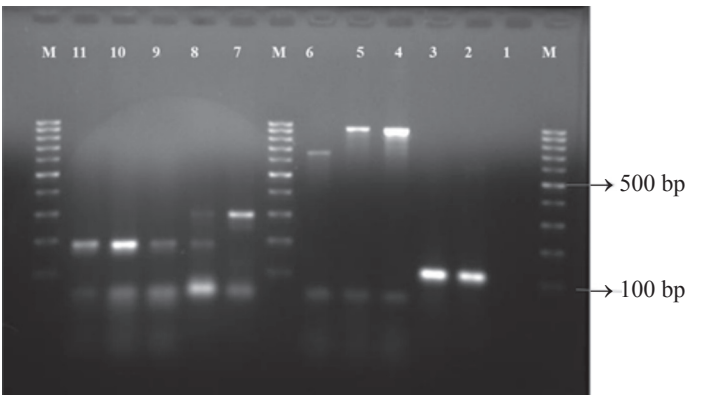

Fig. 1. Electrophorese image of *Enterococcus* spp., *E. fecalis*, *E. faecium*, *E. hirae*, and *E. durans* strains. [M: 100 bp DNA marker (Bioron); 1: Negative control; 2 and 3: *Enterococcus* spp. (112 bp); 4 and 5: *E. faecalis* (941 bp); 6: *E. faecium* (658 bp); 7: *E. durans* (295 bp); 8: *E. hirae*+*E. durans* (mixed culture); 9-11: *E. hirae* (187 bp)].

Of the isolated enterococci, 90 were recovered from cheese samples, 63 from ground beef, 51 from raw cow's milk, and 42 from broiler wing meat. High levels of contamination with *Enterococcus* spp. was detected in cheese samples (90/246, 36.5%), followed by ground beef (63/246, 25.6%), raw milk (51/246, 20.7%), and broiler meat (42/246, 17%). Among the 246 enterococci isolated, 58.13% were determined as VSE and 41.86% were VRE. VSE contamination was detected at a rate of 65.5% (59/90), 63.4% (40/63), 50.9% (26/51), 42.8% (18/42) in cheese, ground beef, raw milk, and broiler wing meat samples, respectively. In contrast, VRE contamination was detected more frequently in broiler meat (24/42, 57.1%), followed by raw milk (25/51, 49%), ground beef (23/63, 36.5%), and cheese (31/90, 34.4%) samples (Table 2).

Table 2. Distribution of *Enterococcus* isolates at species level and in terms of vancomycin resistance

Species	No. of isolates from:											
	Künefe cheese		Ground beef		Broiler meat		Carra cheese		Surk cheese		Raw milk	
	VSE*	VRE**	VSE	VRE	VSE	VRE	VSE	VRE	VSE	VRE	VSE	VRE
E. hirae	0	0	6	1	0	0	0	0	0	0	2	0
E. durans	3	2	0	1	1	0	3	0	3	1	5	0
E. faecalis	0	0	8	1	0	1	1	0	0	0	2	0
E. faecium	2	0	7	6	0	1	2	1	0	0	2	4
E. hirae + *E. durans*	0	0	0	0	0	0	0	0	0	0	1	1
E. faecalis + *E. faecium*	0	0	9	0	0	0	3	0	0	0	2	0
Other enterococcal species	21	17	10	14	17	22	10	1	11	9	12	20
Total	26	19	40	23	18	24	19	2	14	10	26	25

*VSE: Vancomycin-susceptible, **VRE: Vancomycin-resistant

All isolates were identified at species level by multiplex PCR assay and *E. faecium* was the most commonly found species, with a level of 10.1% (25/246), followed by *E. durans* (19/246, 7.7%), *E. faecalis* (13/246, 5.2%) and *E. hirae* (9/246, 3.6%). One hundred and sixty-four of those isolates (66.6%) were detected as other enterococcal species. *E. faecalis, E. faecium* and *E. hirae* were isolated more frequently from ground beef and raw milk, while *E. durans* was isolated more frequently from cheese samples. Also, among the isolates, 6.5% (16/246) were found as mixed cultures (*E. hirae* + *E. durans* and *E. faecalis* + *E. faecium*) that were detected in raw milk, ground beef, and Carra cheese samples (Table 2).

Only pure cultures of isolates *E. faecalis, E. faecium, E. hirae* and *E. durans* were analysed for the presence of virulence genes by multiplex PCR (Fig. 2), while mixed cultures of enterococcal isolates were excluded. In the present study, *gelE* was the predominant (25/66, 37.8%) virulence factor in the isolates, followed by *asa1* (22/66, 33.3%), *esp* (12/66, 18.1%), and *cylA* (4/66, 6.0%). However, none of the isolates were harbouring *hyl* gene. Virulence genes were more frequent in *E. faecalis* than in *E. faecium, E. durans*, and *E. hirae*. Also, in the present study, some of the isolates carried more than one virulence gene, as shown in Table 3.

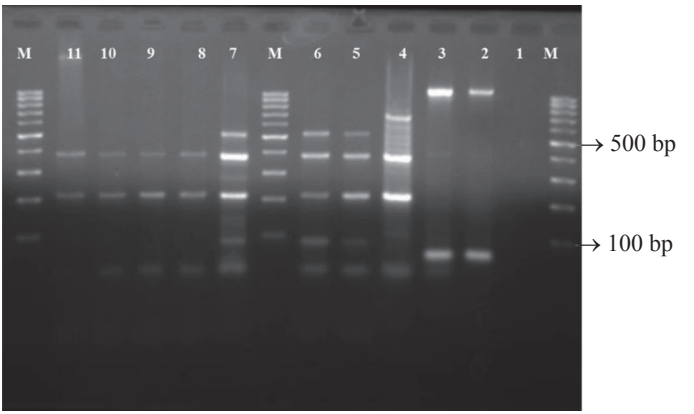

Fig. 2. PCR analysis of the virulence and vancomycin resistance genes of the enterococci isolates. M: 100 bp DNA marker (Bioron); 1: Negative control; 2 and 3: *van A*(+) isolates (1030 bp); 4: *gelE* (213 bp)+*asa1* (375 bp)+*cylA* (688 bp) positive isolate; 5, 6 and 7: *gelE+asa1+esp* (510 bp) positive isolates; 8-11: *gelE+asa1* positive isolates.

Table 3. Occurence of virulence genes among the *E. faecalis, E. faecium, E. hirae* and *E. durans* strains

Isolate (n)	Virulence genes									
	asa1	*gelE*	*asa1 +gelE*	*asa1 +esp*	*gelE +esp*	*cylA +esp*	*asa1+ gelE+esp*	*asa1+ gelE+cylA*	*asa1+gelE +cylA+esp*	*None*
E. faecium (25)	1	2	2	-	1	-	2	-	-	17
E. durans (19)	1	1	2	-	2	1	1	1	-	10
E. faecalis (13)	-	1	3	1	-	-	2	1	1	4
E. hirae (9)	-	-	3	1	-	-	-	-	-	5
Total (66)	2	4	10	2	3	1	5	2	1	36

A total of 230 *Enterococcus* isolates were phenotypically tested for their antibiotic resistance profiles, using the disc diffusion method, and the results were evaluated according to CLSI (2013). However, 16 isolates which were detected as mixed cultures were not analysed by the antibiotic susceptibility test. In this study, all and/or the majority of the *Enterococcus* isolates tested were found to be resistant to ampicillin, rifampicin,

vancomycin, and erythromycin. The most effective antibiotic was gentamicin, because 100% of the isolates were susceptible to it. Also, 98.2%, 81.7%, and 72.1% of the isolates were susceptible to streptomycin, chloramphenicol, and ciprofloxacin, respectively (Table 4).

Table 4. Antimicrobial susceptibility of *Enterococcus* isolates obtained from raw cow's milk, ground beef, broiler wing meat, and traditional cheese samples

	E. faecalis (n = 13)			*E. faecium* (n = 25)			*E. hirae* (n = 9)			*E. durans* (n = 19)			Other enterococcal species (n = 164)		
Antibiotic	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S
Ampicillin	12	0	1	25	0	0	7	0	2	19	0	0	162	0	2
Rifampicin	12	1	0	25	0	0	9	0	0	19	0	0	158	4	2
Vancomycin	12	1	0	25	0	0	9	0	0	18	0	1	150	9	5
Erythromycin	13	0	0	25	0	0	8	1	0	18	1	0	154	10	0
Streptomycin	1	0	12	2	0	23	0	0	9	0	0	19	1	0	163
Chloramphenicol	1	2	10	1	3	21	0	3	6	1	3	15	10	18	136
Ciprofloxacin	2	7	4	4	9	12	0	5	4	0	3	16	7	27	130
Gentamicin	0	0	13	0	0	25	0	0	9	0	0	19	0	0	164

n: number of isolate; R: resistant; I: intermediately resistant; S: susceptible.

In the present study, almost all the *E. faecalis, E. faecium, E. durans*, and *E. hirae* isolates were phenotypically resistant to vancomycin by the disc diffusion method, but vancomycin resistance genes such as *vanB* and *vanC1* genes were not determined in any of the isolates by multiplex PCR. Only three isolates of *E. durans* obtained from Surk cheese samples were found to be carrying the *vanA* gene. Overall, *vanA* gene was detected in 4.5% (3/66) of the isolates tested in our study.

Discussion

In our study, the most frequently isolated species from samples was *E. faecium* and it was found that *E. faecalis* had more virulence genes than other *Enterococcus* species. *E. faecium, E. faecalis,* and *E. hirae* were more often found in raw ground beef and milk, while *E. durans* was generally isolated from cheese. Also, in this study, contamination with *Enterococcus* was most commonly detected in cheese samples.

KASIMOGLU-DOGRU et al. (2010) found that 78% of chicken neck skin is contaminated with *Enterococcus* spp. BUYUKYORUK et al. (2014) found that 73.6% of cheese samples and KOLUMAN et al. (2009) found that 50% of 200 different food samples were contaminated with *Enterococcus* spp.

In contrast to our study, BUYUKYORUK et al. (2014), ÇITAK et al. (2004), KOLUMAN et al. (2009) generally found *E. faecalis* and *E. faecium* in cheese. In our

study, the tested cheeses were traditional and thus, this difference might be due to different cheese production techniques (for example, using raw or pasteurized milk in production).

Similarly, KASIMOGLU-DOGRU et al. (2010) most often isolated *E. faecium* from samples and also observed *E. durans*, *E. faecalis* and *E. hirae*. However, they could not determine either phenotypically or genotypically vancomycin resistance in isolates. Again, the study of ÖZMEN TOĞAY et al. (2010) did not detect *vanB* gene in isolates, similar to our study; but they detected the *vanA* gene in *E. faecalis* strains that were isolated from cheese and sausage. In this study, *vanB* and *vanC1* genes encoding vancomycin resistance were not detected genotypically in any of the isolates, while the *vanA* gene was seen in isolates obtained from Surk cheese. In this context, it may be better to investigate the presence of other genes such as: *vanC2, vanC3, vanD, vanE* and *vanG*, encoding vancomycin resistance in *Enterococcus*.

In accordance with ÖZMEN TOĞAY et al. (2010) and BUYUKYORUK et al. (2014), the *gelE* virulence gene was most commonly detected in isolates, and some isolates carried more than one virulence gene at the same time. SANTESVAN et al. (2015) detected *ace*, *gelE*, *asa* and *cylA* genes with percentages of 68%, 54%, 22% and 4% in enterococci recovered from fecal samples of wild fur seals. GOMES et al. (2008) detected a much higher prevalence of virulence genes in the *E. faecalis* isolates and the *hyl* gene was not determined in any of their isolates, corroborating the results of our study.

In our study, VSE were most frequently isolated from cheese and VRE were most frequently detected in broiler meat. Similarly, KOLUMAN et al. (2009) detected VRE most frequently in chicken meat. When compairing studies done in other countries, consistent with our study, GOMES et al. (2008) reported that cheese and meat samples are generally contaminated with *Enterococcus* and, in those samples, the dominant strain is *E. faecium*. Furthermore, they detected virulence genes at a higher rate in *E. faecalis* when compared with other *Enterococcus* species, and they did not find the *hyl* gene in isolates. Similarly, HAYES et al. (2003) detected *E. faecium*, *E. faecalis,* and *E. hirae* in raw meat samples, in increasing order. ROBREDO et al. (2000), found VRE in chicken meat at a rate of 27.2%, which was lower than in our study. ROBREDO et al. (2000) mentioned that the contamination of foods with VRE can be important for VRE-related infections in humans.

JUNG et al. (2007) determined genes encoding vancomycin resistance (*vanA, vanC1, vanC2*) with PCR, in *Enterococcus* strains that were isolated from different animal resources in Korea, and they reported *E. faecium* carrying the *vanA* gene to be high in poultry. Also, in a study by RADU et al. (2001), poultry samples obtained from local markets in Malaysia were found to be generally contaminated with *E. faecalis*. In addition, *E. durans, E. hirae, E. faecium,* and *E. casseliflavus* were also found in the samples they analized. It was reported that all of the isolates were multidrug resistance

enterococci. *E. faecalis, E. durans, E.hirae,* and *E. faecium* strains were detected as vancomycin-resistant with the disc diffusion method, and all of them were found to have the *vanA* gene genotypically. Similarly, *vanB* and *vanC1* genes were not detected in the isolates.

From the results of the antibiotic susceptibility test, gentamicin was found to be the most effective drug for *Enterococcus* because all the isolates were 100% susceptible to gentamicin. In contrast to our findings, ÇITAK et al. (2004), KASIMOGLU-DOGRU et al. (2010), ÖZMEN TOĞAY et al. (2010), ROBREDO et al. (2000), and HAYES et al. (2003) reported a high-level of aminoglycoside (gentamicin, streptomycin) resistance in their studied isolates. However, ÇITAK et al. (2004) reported high levels of vancomycin and erythromycin resistance among their isolates, which is similar to our findings, whereas KOLUMAN et al. (2009) detected low levels of vancomycin and erythromycin resistance in their study. Again, studies in Brazil (GOMES et al., 2008; FRAZZON et al., 2010), the United States of America (HAYES et al., 2003), and Denmark (AARESTRUP et al., 2000) reported very low levels of vancomycin resistance, or did not detect any resistance in isolates.

In Brazil, GOMES et al. (2008) found that all the *E. faecalis* and *E. faecium* isolates were susceptible to ampicillin, while FRAZZON et al. (2010) found resistant strains. In our study, all *E. faecium* and *E. durans* isolates and 92.3% of *E. faecalis* and 77.7% of *E. hirae* isolates were resistant to ampicillin. In contrast, ampicillin and chloramphenicol were the most effective antibiotics against *Enterococcus* in Malaysia (RADU et al., 2001).

Furthermore, FRAZZON et al. (2010) detected high levels of erythromycin resistance, especially in strains that were isolated from chicken meat, and ascribed this finding to usage of erythromycin in poultry farming for therapeutic purposes. Again, in seals with fur that are seen along the Brazilian coast, antibiotic-resistant *Enterococcus* strains were detected. It was found that these strains had both erythromycin and tetracycline resistance encoding genes and some virulence genes. As a result, these animals are reported to be a reservoir for antibiotic-resistant *Enterococcus* and could play role in the environmental spread of these strains (SANTESTEVAN, 2015).

In Denmark, chloramphenicol resistance was widely found among the *E. faecalis* and *E. faecium* strains isolated from stool samples of people with diarrhea, and broilers and pigs. All the human isolates were reported to be susceptible to vancomycin, and the strains obtained from the two other sources (broilers and pigs) were reported to be resistant at a ratio of 10% and 17%, respectively. Differently, all vancomycin-resistant strains were found to have the *vanA* gene (AARESTRUP et al., 2000).

A study was conducted in Europe in 1997 to determine the vancomycin resistance of *Enterococcus* strains isolated from humans, and the presence of genes encoding this resistance. In this context, 4208 clinical isolates were collected from 27 different countries,

including Turkey. VRE strains carrying the *vanA* gene were most commonly found in England, with a ratio of 2.7%, and isolates with the *vanB* gene were most commonly seen in Slovenia, with a ratio of 2%. Isolates carrying the *vanC* gene were most frequently found in Turkey and Latvia, with ratios of 11.7% and 14.3%, respectively. The highest gentamicin resistance was observed in clinical isolates obtained from Turkey and Greece (SCHOUTEN et al., 2000). In contrast, the *vanC* gene was not found in any of the isolates that were obtained from foodstuffs in our study. Interestingly, all of the isolates obtained from foodstuffs in our study were found to be susceptible to gentamicin. On this basis, we can say that the antibiotic resistance profiles of clinical isolates and *Enterococcus* isolates obtained from foodstuffs might show phenotypic and genotypic differences.

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SAŽETAK

Najčešće dokazana vrsta u ovom istraživanju bila je *Enterococcus faecium* s učestalošću od 10,1 %, zatim *Enterococcus durans* (19/246, 7,7 %), *Enterococcus faecalis* (13/246, 5,2 %) te *Enterococcus hirae* (9/246, 3,6 %). U izolatima spomenutih vrsta pretežno je dokazan gen *gelE* za virulenciju (25/66, 37,8 %), zatim gen *asa1* (22/66, 33,3 %), *esp* (12/66, 18,1 %) te *cylA* (4/66, 6,0 %). Ni u jednog od izolata nije bio ustanovljen gen *hyl*. Gotovo svi ili većina izolata bila je otporna na ampicilin, rifampicin, vankomicin i eritromicin. Ipak, geni za otpornost na vankomicin, kao što su *vanB* i *vanC1*, nisu bili dokazani ni u jednom izolatu višestrukim PCR-om. Samo tri izolata vrste *E. durans*, izdvojena iz surk sira, posjedovala su gen *vanA*. Rezultati pokazuju da se virulentni sojevi enterokoka i sojevi otporni na antimikrobne tvari pojavljuju u hrani životinjskog podrijetla u Turskoj.

Ključne riječi: otpornost na antibiotike; sir; *Enterococcus*; gen *tuf*; virulencija