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INVESTIGATION OF ANTIBIOTIC RESISTANCE GENES IN *ENTEROCOCCUS* STRAINS ISOLATED FROM SUCUK, A TRADITIONAL DRY-FERMENTED TURKISH SAUSAGE*

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ABSTRACT
Enterococcus faecium and *Enterococcus faecalis* are lactic acid bacteria frequently found in fermented meat products such as sausage. These bacteria are important in the fermentation process because they help shape the unique taste, texture, and shelf life of the product. Although some enterococci are known to cause infections, enterococci isolated from various foods, especially meat products, have been shown to have much lower pathogenic potential compared to clinical strains. This has increased interest in foodborne enterococci. In this study, the presence of antibiotic resistance genes for erythromycin (*ermA*, *ermB*, *ermC*), tetracycline (*tetM*, *tetL*), ciprofloxacin (*gyrA*), streptomycin (*strA*, *strB*, *aadA*, *aadE*), vancomycin (*vanA*, *vanB*), and gentamicin [*(aac(6')aph(2")*, *aac(3")II*, *aac(3")IV)*] was investigated in a total of 25 *Enterococcus* strains—*E. faecium* (24) and *E. faecalis* (1) —isolated from traditionally fermented sucuk samples by polymerase chain reaction (PCR). As a result of PCR analysis, the presence of the genes *ermA*, *ermB*, *ermC*, *tetM*, *tetL*, *gyrA*, *strA*, *strB*, *aadA*, *aadE*, *vanA*, *vanB*, *aac(6')aph(2")*, *aac(3")II*, and *aac(3")IV* was not detected in any isolate. Although previous disk diffusion tests indicated that certain strains were resistant to ciprofloxacin (13/25) and erythromycin (1/25), it was observed that these strains did not harbor the corresponding antibiotic resistance genes. *Enterococcus* species are generally regarded as having a questionable status in terms of food safety. In this study, the absence of the investigated antibiotic resistance genes in enterococci isolated from sausage provides a potential advantage for these strains with respect to food safety.
Keywords: Sausage, *Enterococcus,* antibiotic resistance, polymerase chain reaction

GELENEKSEL KURU FERMENTE TÜRK SUCUĞUNDAN İZOLE EDİLEN ENTEROKOK SUŞLARINDA ANTİBİYOTİK DİRENÇ GENLERİNİN ARAŞTIRILMASI

ÖZ
Enterococcus faecium ve *Enterococcus faecalis*, sucuk gibi fermente et ürünlerinde sıklıkla bulunan laktik asit bakterisi türleridir. Bu bakteriler, ürünün benzersiz tadını, dokusunu ve raf ömrünü şekillendirmeye yardımcı olmaları nedeniyle fermentasyon sürecinde önem arz etmektedir. Bazı enterokokların enfeksiyonlara yol açtığı bilinse de çeşitli gıdalardan, özellikle et ürünlerinden izole edilen

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enterokokların, klinik suşlara kıyasla çok daha düşük patojenik potansiyele sahip olduğu gösterilmiştir. Bu durum, gıda kaynaklı enterokoklara olan ilgiyi artırmıştır. Bu çalışmada, geleneksel olarak fermente edilmiş sucuk örneklerinden izole edilen *E. faecium* (24 adet) ve *E. faecalis* (1 adet) olmak üzere toplam 25 *Enterococcus* suşunda eritromisin (*ermA, ermB, ermC*), tetrasiklin (*tetM*, *tetL*), siprofloksasin (*gyrA*), streptomisin (*strA, strB, aadA, aadE*), vankomisin (*vanA*, *vanB*) ve gentamisin [*(aac(6')aph(2''), aac(3'')II, aac(3'')IV)*] direnç genlerinin varlığı polimeraz zincir reaksiyonu (PZR) ile araştırılmıştır. PZR analizinin sonucunda, hiçbir izolatta *ermA, ermB, ermC, tetM, tetL, gyrA, strA, strB, aadA, aadE, vanA, vanB, aac(6')aph(2''), aac(3'')II* ve *aac(3'')IV* genlerinin varlığı tespit edilmemiştir. Daha önce yapılan disk difüzyon testlerine göre bazı suşların siprofloksasin (13/25) ve eritromisine (1/25) dirençli olduğu bilinmesine rağmen bu suşların da söz konusu antibiyotik direnç genlerini içermediği görülmüştür. Enterokok türleri genellikle gıda güvenliği açısından şüpheli bir statüye sahip olarak değerlendirilir. Yapılan çalışmada araştırılan antibiyotik direnç genlerinin sucuktan izole edilen enterokoklarda bulunmaması bu suşlara gıda güvenliği açısından bir avantaj sağlamaktadır.
Anahtar kelimeler: Sucuk, *Enterococcus,* antibiyotik direnç, polimeraz zincir reaksiyonu

INTRODUCTION

Enterococci, members of lactic acid bacteria, can grow in environments with a temperature range of 10-45°C, up to pH of 9.6, and 6.5% NaCl. Remarkably, enterococci are microorganisms that can colonize various ecosystems such as plants, soil, water, the gastrointestinal systems of humans, animals, and poultry, and can develop under adverse environmental conditions. This ability of enterococci to grow under extreme environmental conditions can lead to contamination of carcasses and meat during slaughter. The resistance of enterococci to extreme environmental conditions leads to a high ability of these bacteria to spread throughout the food chain (Foulquié Moreno et al., 2006; Byappanahalli et al., 2012; Cassenego et al., 2017). Enterococci, which can be found in fermented food products such as cheese, sausage, olives, and vegetables, are believed to play an important role, especially in traditional cheese and meat products, due to their unique aroma, texture, flavor, and taste (Graham et al., 2020; Dapkevicius et al., 2021). Moreover, certain foodborne enterococcal species, which produce bacteriocins, have the potential to act as natural food preservatives in food systems and have recently attracted the attention of researchers due to their probiotic characteristics. Despite the technological benefits of enterococci in traditional fermented food products and their positive impact on consumer health, the safety of these bacteria remains a topic of debate, particularly due to their potential to contain antibiotic resistance and virulence factors, and/or facilitate their transfer (Graham et al., 2020). Due to the uncertainty regarding their safety, enterococci are not included in the European Food Safety Authority's Qualified Presumption of Safety (QPS) list or in the United States under the "Generally Recognized as Safe" (GRAS) status. The lack of a recognized safety status for enterococci has prevented the use of these bacteria as industrial food cultures, despite their potential benefits (Dapkevicius et al., 2021). Some enterococci are considered opportunistic human pathogens that cause hospital-acquired infections such as endocarditis, bacteremia, and urinary tract infections. Enterococcal infections are primarily associated with *E. faecalis* and *E. faecium*. Enterococci can be resistant to a wide range of antibiotics commonly used in human treatment, as well as those used in animal treatment, prophylaxis, or growth promotion. Although antibiotic resistance is not a virulence factor in itself, the presence of multidrug resistance in enterococci is a contributing factor to their pathogenicity. Resistant strains can persist in the host due to their insensitivity to antimicrobial treatments, leading to therapeutic failure and increasing the duration and severity of infections. Moreover, these strains often encode various virulence factors such as adhesion, biofilm formation, and immune evasion, which act synergistically to reinforce the invasiveness and treatment-resistant nature of infections. In this context, antibiotic resistance is regarded as one of the principal factors contributing to the clinical significance of enterococci, particularly in nosocomial infections (Ben Braïek and Smaoui 2019, Khalifa et al., 2024).

Enterococci have both intrinsic and acquired antibiotic resistance, which is encoded on chromosomes and plasmids or transposons, respectively (Demirgül and Tuncer, 2017). Acquired antibiotic resistance genes can be horizontally transferred between distant or closely related bacteria via mobile genetic elements. In recent years, there has been an increase in studies aimed at detecting antibiotic resistance in non-pathogenic bacteria, as they serve as reservoirs for antibiotic resistance genes (Talon and Leroy, 2011). Enterococci are naturally resistant to commonly used antimicrobial compounds, such as β-lactams, cephalosporins, and aminoglycosides, to varying degrees, which hampers the treatment of enterococcal infections. Similarly, the presence of acquired antibiotic resistance profiles in these bacteria is of significant concern. Due to their ability to acquire foreign genetic material, including transposons and plasmids, enterococci rapidly became resistant to additional antimicrobial agents, such as erythromycin and tetracyclines, shortly after their introduction into clinical practice (Semedo-Lemsaddek et al., 2021).

To date, researchers have focused on the presence of these bacteria in raw materials prepared for further processing (e.g., through heat treatment of raw meat), while there has been less research on the presence of antibiotic-resistant enterococci in ready-to-eat foods sold in retail chains, such as smoked meats, sausages, fermented salami, offal products, formed meat products, and canned foods. The detection of *Enterococcus* strains in various foods and the study of their antibiotic resistance and the genes encoding resistance to different antibiotics will enable risk assessment and the selection of the appropriate strategy for food inspection. Therefore, the aim of this study is to determine the presence of antibiotic resistance genes in enterococci isolated from traditionally produced sucuk.

MATERIAL AND METHODS

Material

In the study, a total of 25 *Enterococcus* strains, including 24 *E. faecium* and 1 *E. faecalis* isolated from traditionally fermented sucuk samples, were used, and their antibiotic resistance profiles were determined by the disk diffusion method (CLSI, 2012; Yüceer and Özden Tuncer, 2015). The antibiotic disk diffusion profiles of the enterococcal strains are given in Table 1. For the investigation of the presence of antibiotic resistance genes in the enterococcal strains using PCR, control strains were used: *E. casseliflavus*/*E. gallinarum* DYE44 (*ermA*$^+$, *ermB*$^+$, *gyrA*$^+$, *aadA*$^+$), *E. gallinarum* DYE45 (*ermA*$^+$, *tetM*$^+$, *tetL*$^+$), *E. gallinarum* DYE46 (*strA*$^+$) (Akpınar Kankaya and Tuncer, 2020), *E. faecium* FYE2 (*ermC*$^+$) (Demirgül and Tuncer, 2017), and *E. faecalis* ATCC29212 (*tetM*$^+$), *E. faecium* ATCC51559 (*ermB*$^+$, *aac6'aph2''*$^+$, *vanA*$^+$), and *E. faecalis* ATCC51299 (*aac6'aph2''*$^+$, *vanB*$^+$), which were obtained from the bacterial genetic culture collection of the Food Engineering Department at Süleyman Demirel University. The stock cultures of the enterococcal strains used in the study were cultivated in de Man Rogosa and Sharpe (MRS, LAB M, UK) broth with two successive passages at 37°C for 18 hours and preserved at 4°C.

Methods

Isolation of genomic DNA

To isolate genomic DNA, 25 enterococcal strains were cultured in MRS broth (LAB M) at 37°C for 18 hours. From these active cultures, 500 µL was transferred into sterile Eppendorf tubes, which were then centrifuged at 13,000 rpm for 5 minutes (Sigma 2-16P, Germany) to pellet the cells. The supernatant was discarded, and 500 µL of lysis buffer (pH 8.0 ± 0.02) was added to the resulting cell pellets. The pellets were resuspended by vortexing. The tubes were subsequently incubated in a water bath at 37°C for 30 minutes. After incubation, 30 µL of a 10% (w/v) sodium dodecyl sulfate (SDS, Serva, Heidelberg, Germany) solution was added, and the tubes were heated in a water bath at 80°C for 5 minutes (Nüve NB9, Türkiye). Following lysis, 700 µL of a phenol-chloroform solution (Merck, Germany), prepared at a 1:10 (v/v) ratio, was added to the suspension. The tubes were centrifuged at 13,000 rpm for 5 minutes (Sigma 2-16P, Germany), and the upper aqueous phase was transferred to new sterile Eppendorf tubes using a micropipette. To this phase, 700 µL of 2-propanol (Merck, Germany) was added, and the tubes were centrifuged again

at 13,000 rpm for 5 minutes (Sigma 2-16P, Germany). The supernatant was discarded, and the DNA pellets were air-dried before being resuspended in 50 µL of Tris-EDTA buffer (pH 8.0 ± 0.02). The resulting genomic DNA samples were stored at -20°C, following the protocol described by Cancilla et al. (1992).

Table 1. Antibiotic disk diffusion profiles of enterococcal strains

Strains	Antibiotics*					
	VA	S	CIP	TE	CN	E
E. faecium OBS3	S**	S	R	S	S	I
E. faecium OBS4	S	S	S	S	S	S
E. faecium OBS11	S	S	I	S	S	I
E. faecium OBS12	S	S	S	S	S	R
E. faecium OBS13	S	S	I	S	S	I
E. faecium OBS14	S	S	I	S	S	S
E. faecium OBS15	S	S	I	S	S	I
E. faecalis OBS18	S	S	R	S	S	I
E. faecium OBS20	S	S	R	S	S	I
E. faecium OBS23	S	S	S	S	S	I
E. faecium OBS24	S	S	I	S	S	I
E. faecium OBS25	S	S	R	S	S	I
E. faecium OBS26	S	S	R	S	S	I
E. faecium OBS29	S	S	R	S	S	I
E. faecium OBS31	S	S	R	S	S	I
E. faecium OBS32	S	S	I	S	S	I
E. faecium OBS33	S	S	R	S	S	I
E. faecium OBS34	S	S	I	S	S	I
E. faecium OBS37	S	S	R	S	S	I
E. faecium OBS39	S	S	R	S	S	I
E. faecium OBS41	S	S	I	S	S	I
E. faecium OBS45	S	S	R	S	S	I
E. faecium OBS46	S	S	I	S	S	I
E. faecium OBS47	S	S	R	S	S	I
E. faecium OBS48	S	S	R	S	S	I

*VA, vancomycin (30 µg); S, streptomycin (300 µg); CIP, ciprofloxacin (5 µg); TE, tetracycline (30 µg); CN, gentamicin (120 µg); E, erythromycin (15 µg).
** S, susceptible; I, intermediary; R, resistant.

Agarose gel electrophoresis of genomic DNA samples

Genomic DNA samples were subjected to electrophoresis on a 1% (w/v) agarose gel (AppliChem, Darmstadt, Germany) using the Thermo OWL EASYCAST B2 horizontal gel electrophoresis system (United States). A 10 µL aliquot of each sample was mixed with 2 µL of loading dye and loaded into the wells. Electrophoresis was performed at 85 V for 1.5 to 2 hours with tris-acetate electrophoresis buffer. Following electrophoresis, the gel was stained for 60 minutes with ethidium bromide solution (0.2 µg/mL). After staining, the gel was visualized under ultraviolet light at a wavelength of 312 nm, and an image was captured using a Nikon D5100 digital camera.

Investigation of antibiotic resistance genes by PCR

The primer pairs, PCR protocols, and PCR product sizes employed for the detection of antibiotic resistance genes in enterococcal strains, including those for erythromycin (*ermA, ermB, and ermC*), tetracycline (*tetM* and *tetL*), ciprofloxacin (*gyrA*), streptomycin (*strA, strB, aadA, and aadE*), vancomycin (*vanA* and *vanB*), and gentamicin (*aac(6')aph(2"), aac(3")II*, and *aac(3")IV*), are

detailed in Table 2. PCR products were subjected to electrophoresis on a 1.5% (w/v) agarose gel, using the O'GeneRuler 100-bp DNA ladder (Fermentas) as a molecular size marker. DNA bands were stained with ethidium bromide and visualized under ultraviolet illumination, followed by photographic documentation using a Nikon D5100 digital camera.

Table 2. Primers and product sizes used for the detection of antibiotic resistance genes

Antibiotics	Gene	Primer Sequence (5'-3')	Amplicon size (bp)	References
Erythromycin	*ermA*	AAGCGGTAAAACCCCTCTGAG TCAAAGCCTGTCGGAATTGG	441	Ouoba et al., 2008
	ermB	CATTTAACGACGAAACTGGC GGAACATCTGTGGTATGGCG	425	Ouoba et al., 2008
	ermC	ATCTTTGAAATCGGCTCAGG CAAACCCGTATTCCACGATT	295	Ouoba et al., 2008
Tetracycline	*tetM*	GTTAAATAGTGTTCTTGGAG CTAAGATATGGCTCTAACAA	657	Ouoba et al., 2008
	tetL	GTTGCGCGCTATATTCCAAA TTAAGCAAACTCATTCCAGC	788	Ouoba et al., 2008
Ciprofloxacin	*gyrA*	GAYTATGCWATGTCAGTTATTGT GGAATRTTRGAYGTCATACCAAC	286	Ouoba et al., 2008
Streptomycin	*strA*	CTTGGTGATAACGGCAATTC CCAATCGCAGATAGAAGGC	546	Ouoba et al., 2008
	strB	ATCGTCAAGGGATTGAAACC GGATCGTAGAACATATTGGC	509	Ouoba et al, 2008
	aadA	ATCCTTCGGCGCGATTTTG GCAGCGCAATGACATTCTTG	282	Ouoba et al., 2008
	aadE	ATGGAATTATTCCCACCTGA TCAAAACCCCTATTAAAGCC	565	Ouoba et al., 2008
Vancomycin	*vanA*	GGGAAAACGACAATTGC GTACAATGCGGCCGTTA	732	Dutka Malen et al., 1995
	vanB	ACGGAATGGGAAGCCGA TGCACCCGATTTCGTTC	647	Depardieu et al., 2004
Gentamicin	*aac(6')aph(2")*	CCAAGAGCAATAAGGGCATA CACTATCATAACCACTACCG	220	Ouoba et al., 2008
	aac(3")II	TGAAACGCTGACGGAGCCTC GTCGAACAGGTAGCACTGAG	369	Ouoba et al., 2008
	aac(3")IV	GTGTGCTGCTGGTCCACAGC AGTTGACCCAGGGCTGTCGC	627	Ouoba et al., 2008

RESULTS AND DISCUSSION

Genomic DNA isolation and agarose gel electrophoresis

The agarose gel electrophoresis image of the genomic DNA samples from the enterococcal strains is presented in Figure 1.

Investigation of antibiotic resistance genes by PCR

PCR analysis aimed at detecting erythromycin resistance genes revealed the absence of *ermA*, *ermB*, and *ermC* genes in all 25 enterococcal strains (Figure 2). A comparison between the genotypic PCR results and phenotypic resistance profiles showed a correlation only in the *E. faecium* strains OBS4 and OBS14, which were phenotypically sensitive to erythromycin. Conversely, no correlation was observed between the PCR results and the disk diffusion profiles of the phenotypically erythromycin-resistant *E. faecium* OBS12 strain or the remaining 22 enterococcal strains, which exhibited moderate resistance. The absence of *ermA*, *ermB*, and *ermC* genes in these strains suggests that alternative mechanisms may contribute to the observed resistance. Consistent with our findings, Hummel et al. (2007) reported that enterococci strains isolated from cheese samples, which exhibited phenotypic resistance to erythromycin, did not harbor any of the *ermA*, *ermB*, or *ermC* genes, as determined by PCR

analysis. Similarly, Jahan et al. (2013) observed that enterococcal strains isolated from fermented meat and meat products displayed phenotypic resistance to erythromycin, yet lacked the presence of the *ermA*, *ermB*, and *ermC* genes. In a study by Demirgül and Tuncer (2017), enterococcal strains isolated from sausage samples exhibited resistance to erythromycin according to the disk diffusion test; however, PCR analysis revealed no presence of the *ermA*, *ermB*, or *ermC* genes. Recently, Geniş et al. (2024) identified erythromycin resistance in *E. mundtii* (1) and *E. faecium* (1) strain isolated from small ruminant colostrum, among a total of *E. mundtii* (11) and *E. faecium* (2) strains. Nevertheless, none of the isolates contained the *ermA*, *ermB*, or *ermC* resistance genes. Macrolide antibiotics, such as erythromycin, are commonly employed in the treatment of respiratory infections, including community-acquired pneumonia, bronchitis, laryngitis, Legionnaire's disease, and whooping cough (Yamagami et al., 2024). Given the potential for the horizontal transfer of erythromycin resistance genes via mobile genetic elements, the absence of *erm* genes in the enterococcal strains in this study represents a positive finding.

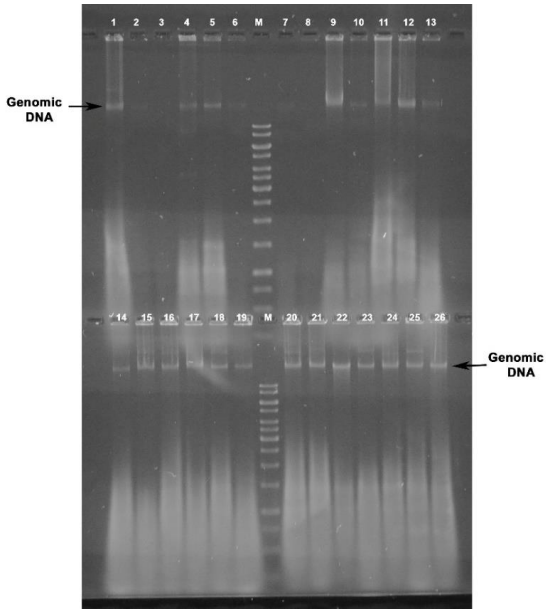

Figure 1. Agarose gel electrophoresis image of genomic DNA samples of some *Enterococcus* strains used in this study.

Results from PCR trials demonstrated that enterococcal strains, known to be phenotypically sensitive to tetracycline, did not harbor the *tetM* and *tetL* genes (Figure 3). These findings, derived from PCR analysis, corroborate the tetracycline disk diffusion test results previously reported. Hummel et al. (2007) observed that *E. faecium* and *E. faecalis* strains isolated from milk and cheese primarily contained the *tetL* gene, followed by the *tetM* gene. Moreover, they reported that 56% of the tested strains exhibited *tetK* resistance, while neither the *tetO* nor the *tetS* genes were detected in any of the strains. In a study conducted by Demirgül and Tuncer (2017), the presence of the *tetM* gene was identified in some tetracycline-sensitive *E. faecium* strains isolated from sausage, whereas others harbored the *tetL* gene. Additionally, consistent with our findings, they reported that none of the tetracycline resistance genes (*tetM*, *tetL*, *tetS*, *tetK*, or *tetO*) were present in *E. faecium* and *E. faecalis* strains identified as phenotypically sensitive or moderately resistant. Golob et al. (2019) reported a phenotypic tetracycline resistance rate of 29.2% in *E. faecalis* strains isolated from fresh pork and beef. Tetracyclines are a widely utilized class of

antibiotics, valued for their broad spectrum of activity and relative affordability compared to other antimicrobial agents. For many years, tetracyclines have been routinely added to animal feed at sub-therapeutic doses as growth promoters. However, prolonged use of tetracyclines has been linked to adverse effects, including allergic reactions in both humans and animals, as well as alterations in environmental microbiota and bacterial populations. Exposure to environmental stressors induces bacterial cells to adapt by regulating specific molecular mechanisms. These mechanisms are often accompanied by the development of cross-resistance, heightened resistance to antimicrobial agents, upregulation of particular gene groups, or the acquisition of antibiotic resistance genes via horizontal gene transfer (Giacometti et al., 2021; Wiśniewski et al., 2024). In this context, the absence of detectable transferable tetracycline resistance genes in enterococcal strains represents a significant advantage.

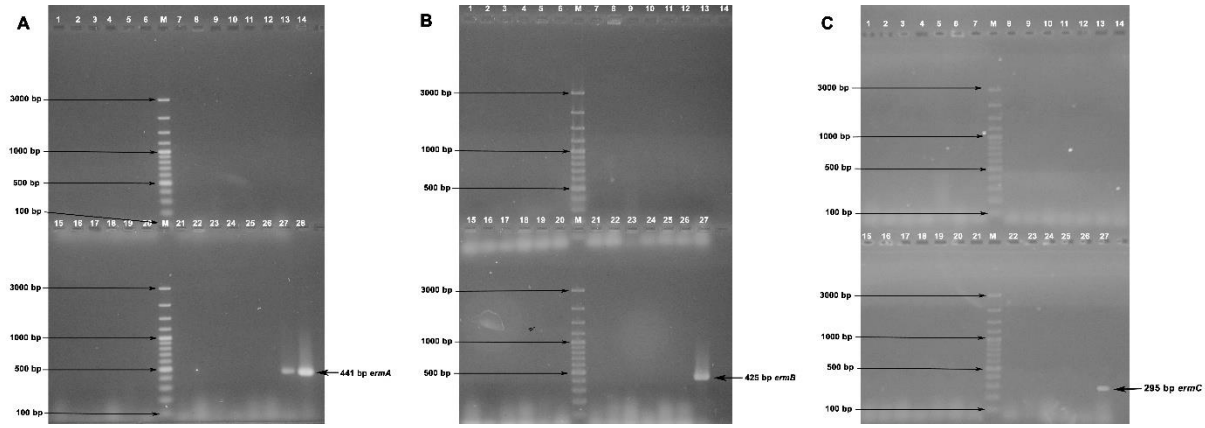

Figure 2. PCR amplification of *ermA* (A), *ermB* (B), and *ermC* (C) structural genes in *Enterococcus* strains (lines 2-26). Line 1: negative control (water) and line M: DNA ladder (O'GeneRuler™ 100-bp DNA ladder, Fermentas #SM1153, Lithuania). For panel A, line 27 contains *E. casseliflavus*/*E. gallinarum* DYE44 (positive control), and line 28 contains *E. gallinarum* DYE45 (positive control). For panel B, line 27 contains *E. faecium* ATCC 51559 (positive control). For panel C, line 27 contains *E. faecium* FYE2 (positive control).

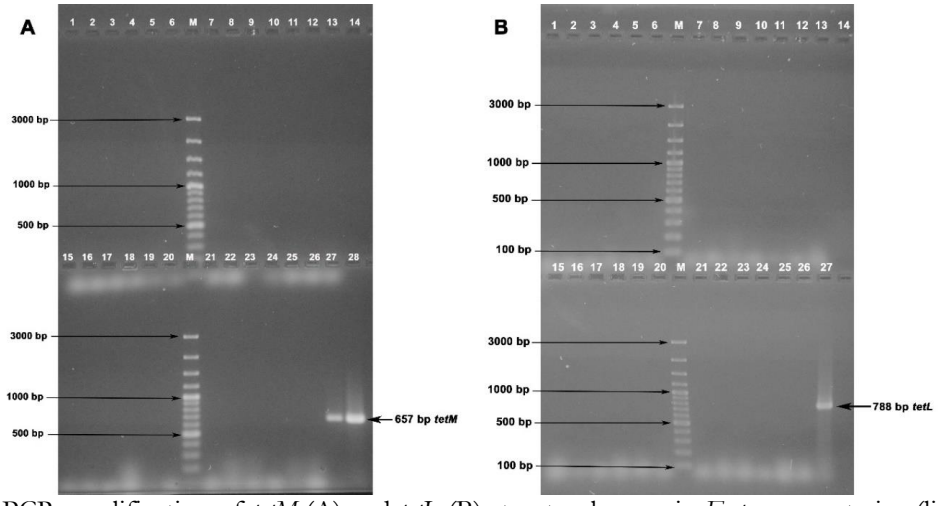

Figure 3. PCR amplification of *tetM* (A) and *tetL* (B) structural genes in *Enterococcus* strains (lines 2-26). Line 1: negative control (water) and line M: DNA ladder (O'GeneRuler™ 100-bp DNA ladder, Fermentas #SM1153, Lithuania). For panel A, line 27 contains *E. faecium* ATCC 29212 (positive control), and line 28 contains *E. gallinarum* DYE45 (positive control). For panel B, line 27 contains *E. gallinarum* DYE45 (positive control).

PCR testing targeting the *gyrA* gene, associated with ciprofloxacin-specific resistance, revealed that not only phenotypically sensitive *E. faecium* strains (OBS4, OBS12, and OBS23), but also all resistant or moderately resistant enterococcal strains, lacked the *gyrA* gene. This finding aligns with the results reported by Jahan et al. (2013), who found that while enterococcal strains isolated from fermented meat and meat products exhibited phenotypic resistance to ciprofloxacin, they did not harbor the *gyrA* gene. Similarly, Demirgül and Tuncer (2017) reported a study conducted in Türkiye, where phenotypically ciprofloxacin-resistant enterococcal strains (23) were isolated from sucuk; however, only one isolate contained the *gyrA* gene. In recent years, with the increasing use of fluoroquinolones, high-level ciprofloxacin resistance has become more prevalent in clinical *E. faecalis* isolates. Furthermore, ciprofloxacin resistance is now widespread among *E. faecium* and *E. faecalis* strains isolated from poultry (Kim et al., 2018).

In this study, none of the enterococcal strains known to be sensitive to streptomycin (300 µg) contained the *strA*, *strB*, *aadA*, or *aadE* resistance genes, as determined by PCR analysis. These findings are consistent with the previous results obtained from the streptomycin disk diffusion test. Similarly, Geniş et al. (2024) found that all *E. mundtii* (11) and *E. faecium* (2) strains isolated from goat and sheep colostrum were sensitive to streptomycin (300 µg) and did not harbor the streptomycin resistance genes investigated in the present study. Conversely, Delpech et al. (2012) identified streptomycin resistance profiles in *E. faecalis* strains isolated from animal-derived foods, detecting the presence of streptomycin resistance genes in resistant strains through PCR. In the same study, resistance genes were not found in strains that were phenotypically sensitive to streptomycin. Similarly, Ben Said et al. (2016) reported that two of the four *E. faecalis* strains isolated from vegetables were resistant to streptomycin and possessed the *ant(6)* gene among the investigated resistance genes. In another study, Kürekci et al. (2016) detected high-level (300 µg) streptomycin resistance in *E. faecalis* strains isolated from cheese samples. However, they were unable to identify the structural streptomycin resistance gene in both streptomycin-resistant and sensitive strains. Özdemir and Tuncer (2020) found that all high-level streptomycin (300 µg)-resistant enterococcal isolates from traditional cheese samples carried aminoglycoside-modifying resistance genes. Similarly, Yalçın et al. (2023) demonstrated that all high-level streptomycin-resistant enterococcal strains (31) isolated from retail chicken meat harbored aminoglycoside-modifying resistance genes. The acquisition of transferable aminoglycoside-modifying enzyme genes, which code for three enzyme groups—acetyltransferase, phosphotransferase, and nucleotidyltransferase—results in the development of high-level aminoglycoside resistance (Guzman Prieto et al., 2016). Given that streptomycin is a clinically significant aminoglycoside antibiotic, the absence of transferable resistance genes in the enterococcal strains (25) examined in this study represents a positive outcome for public health.

As a result of PCR testing, it was determined that all strains known to be sensitive to vancomycin lacked the vancomycin resistance genes *vanA* and *vanB* (Figure 4). Delpech et al. (2012) identified the presence of the *vanA* gene exclusively in vancomycin-resistant *E. faecium* strains isolated from animal-derived food products. In contrast, similar to the findings of the present study, they were unable to detect the *vanA* and *vanB* genes in vancomycin-sensitive *E. faecalis* strains. Additionally, Demirgül and Tuncer (2017) observed a correlation between phenotypic and genotypic characteristics in *E. faecium* and *E. faecalis* strains isolated from sausages, noting that vancomycin-sensitive strains did not harbor the vancomycin resistance genes. El-Oraby et al. (2023) also reported that *E. faecalis* strains (10) isolated from chicken meat were vancomycin-sensitive and lacked the *vanA* and *vanB* genes. In contrast, Gürler et al. (2024) investigated the presence of *vanA* and *vanB* resistance genes in *E. faecium* (98) and *E. faecalis* (153) strains that were phenotypically sensitive to vancomycin, and found the *vanA* gene in a single *E. faecalis* strain, while the *vanB* gene was absent in all strains. Among vancomycin resistance phenotypes, VanA

(resistance to both vancomycin and teicoplanin) and VanB (resistance to vancomycin only) are the most prevalent. These VanA and VanB phenotypes are commonly observed in veterinary, clinical, and food-derived isolates but are less frequent in environmental or gastrointestinal system isolates (Murray, 1997; Murray, 2000). Vancomycin-resistant enterococci (VRE) have emerged as a growing global concern since their initial identification in the 1980s, and the subsequent report of the *vanA* gene in 1993. In 2017, the World Health Organization (WHO) classified vancomycin-resistant enterococci as a priority pathogen on the "Global Priority List of Antibiotic-Resistant Bacteria," urging immediate attention (WHO, 2017). The use of avoparcin, a vancomycin analog, as a growth-promoting feed additive has been implicated in the rise of vancomycin-resistant enterococci in food animals. Despite a ban on avoparcin use for over 25 years, VRE isolation continues to be reported in pig farms in Denmark (Telli et al., 2021). Although foodborne enterococci are not typically regarded as a direct source of antibiotic-resistant enterococci in humans, they represent a potential risk for the transfer of resistance determinants—such as *van* genes—to enterococcal strains adapted to humans. Consequently, food-producing animals may serve as a reservoir for VRE, with horizontal gene transfer of vancomycin resistance between animal-adapted and human-adapted enterococci or through the clonal spread of resistant strains. For instance, studies have shown that the colonization of vancomycin-resistant enterococcal strains of animal origin within the human gastrointestinal tract can facilitate the transfer of vancomycin resistance genes to both enterococci and other pathogens within the intestinal microbiota via conjugation (Chajęcka-Wierzchowska et al., 2020).

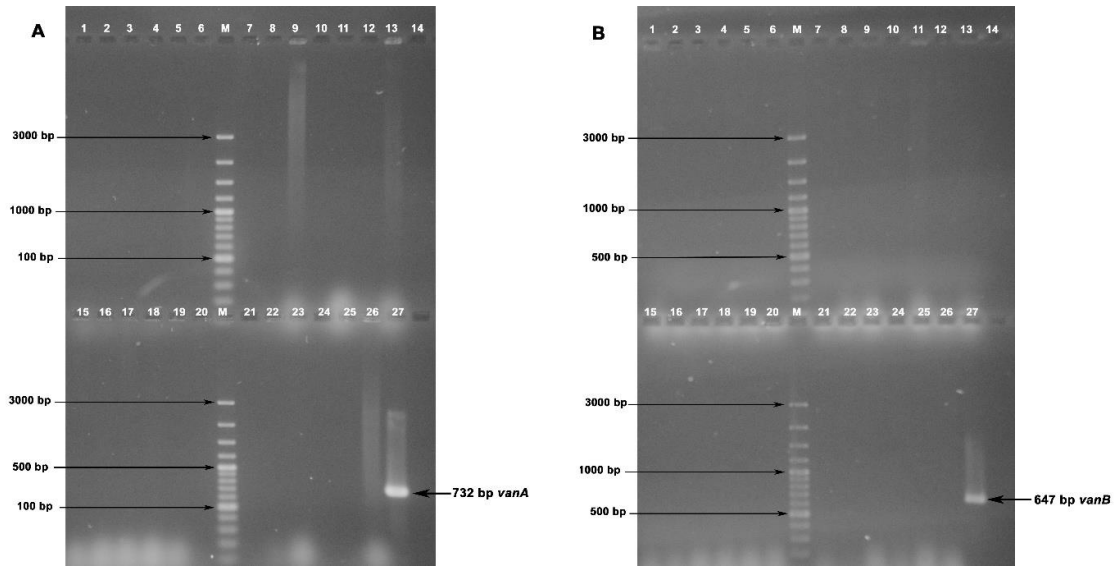

Figure 4. PCR amplification of *vanA* (A) and *vanB* (B) structural genes in *Enterococcus* strains (lines 2-26). Line 1: negative control (water) and line M: DNA ladder (O'GeneRuler™ 100-bp DNA ladder, Fermentas #SM1153, Lithuania). For panel A, line 27 contains *E. faecium* ATCC51559 (positive control). For panel B, line 27 contains *E. faecalis* ATCC51299 (positive control).

PCR experiments demonstrated that none of the strains known to be sensitive to gentamicin harbored the gentamicin resistance genes *aac(6')aph(2'')* (Figure 5), *aac(3')II*, and *aac(3')IV*. Between 2000 and 2002, the number of gentamicin-resistant *E. faecalis* isolates from pigs in Denmark increased by two- to fourfold. Concurrently, there was a rise in the number of *E. faecalis* isolates exhibiting high-level gentamicin resistance in patients with endocarditis infections (DANMAP, 2002). Later, Larsen et al. (2010) reported that all these isolates, both from humans

and pigs, belonged to the same clonal group, with pigs serving as a reservoir for *E. faecalis* strains with high-level gentamicin resistance in enterococcal infections. The specific host preferences of enterococci do not preclude the potential for antimicrobial resistance to be transmitted from animals to humans via enterococci. Indeed, several studies have indicated that high-level gentamicin-resistant enterococcal strains are transmitted from animals to humans through the food chain, with enterococcal strains isolated from both animal-derived foods and humans harboring the same aminoglycoside resistance genes (Sparro et al., 2012; Jaimee and Halami, 2016).

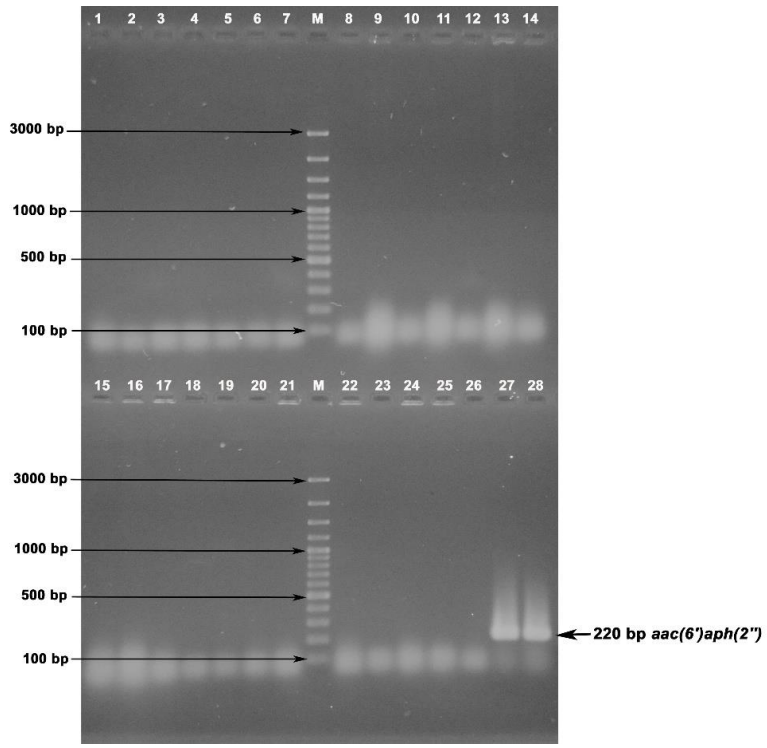

Figure 5. PCR amplification of *aac(6')aph(2")* structural gene in *Enterococcus* strains (lines 2-26). Line 1: negative control (water), line M: DNA ladder (O'GeneRuler™ 100-bp DNA ladder, Fermentas #SM1153, Lithuania), line 27: *E. faecium* ATCC51559 (positive control), and line 28: *E. faecalis* ATCC51299 (positive control).

Enterococci, which are native to the digestive system, can develop resistance as a survival mechanism when exposed to antibiotics. The potential exists for antibiotic-resistant enterococci to spread to humans through direct contact with animals or the consumption of meat products (Shepard and Gilmore, 2002; Kühn et al., 2005). Additionally, because enterococci exhibit high resistance to heat, pH, and salt concentrations, they can survive in fermented or cooked meat products (Shepard and Gilmore, 2002; Teixeira and Facklam, 2003). Antibiotic-resistant enterococci found in farm animals and in meat products derived from these animals harbor a range of natural and acquired resistance mechanisms against antibiotics commonly used in clinical settings, as well as efficient genetic exchange mechanisms that facilitate the spread of these resistance profiles. Antibiotic resistance genes, located on mobile genetic elements, can be transferred to human-derived enterococci (Shepard and Gilmore, 2002; Moubarek et al., 2003; Huys et al., 2013; Lester et al., 2006; Werner et al., 2013). In particular, antibiotic-resistant enterococci acquired through food consumption may transfer their antibiotic resistance genes to

pathogens within the human system. Consequently, enterococci serve as a significant gene pool in the dissemination of antibiotic resistance mechanisms (Chajęcka-Wierzchowska et al., 2021).

Additionally, the spread of antibiotic resistance in enterococci, which are opportunistic pathogens, has led to an increased prevalence of infections caused by these bacteria, particularly among individuals with compromised immune systems. Moreover, the rise in multidrug resistance rates in enterococci has significantly reduced the available antibiotic treatment options for enterococcal infections (Inoglu and Tuncer, 2013; Yogurtcu and Tuncer, 2013; Tuncer et al., 2013; Garrido et al., 2014; Demirgül and Tuncer, 2017).

CONCLUSION

Enterococci possess genetic flexibility that enables them to survive under environmental stress conditions and emerge as significant pathogens in hospital-acquired infections. They can readily acquire and disseminate mobile genetic elements —such as plasmids, phages, and transposons—that carry antibiotic resistance and virulence genes. When combined with their ability to form biofilms and transfer resistance genes to other bacteria, this capacity renders them clinically concerning pathogens. Notably, *E. faecalis* and *E. faecium* have adapted to hospital settings through horizontal gene transfer, leading to the emergence of resistant strains. This adaptability complicates treatment strategies and contributes to increased mortality rates, particularly in cases involving vancomycin-resistant enterococci (VRE). In the conducted study, the presence of structural resistance genes for erythromycin, gentamicin, ciprofloxacin, streptomycin, tetracycline, and vancomycin in *Enterococcus* strains isolated from sucuk was investigated using PCR. The results revealed the absence of the targeted antibiotic resistance genes in the *Enterococcus* strains, indicating that these strains do not pose a risk of serving as reservoirs for the dissemination of antibiotic resistance mechanisms. This finding presents an advantage for the potential use of these strains as starter, adjunct starter, or probiotic cultures. However, future studies should assess the virulence factors of these strains through both phenotypic and genotypic methods. Subsequently, strains that lack virulence properties should be further investigated for their industrial and probiotic characteristics, with an emphasis on evaluating their potential for application in the food industry.

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CONFLICT OF INTEREST

The authors have declared no conflicts of interest for this article.

AUTHOR CONTRIBUTIONS

İbrahim Hakan Falak: investigation, data curation, writing – original draft; Banu Özden Tuncer: methodology, conceptualization, investigation, resources, supervision, project administration, funding acquisition, writing – review & editing. All authors read and approved the final manuscript.

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