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# Detection of vancomycin resistance genes with polymerase chain reaction in enterococci isolated from mastitis infection in cows

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**ABSTRACT**

This study aimed to determine the prevalence of enterococci and the presence of vancomycin resistance genes in the context of mastitis infections, which cause economic losses and reduced yield in dairy cows in the Balıkesir province. This is the most comprehensive study conducted in Turkey and Europe on vancomycin resistance in enterococci, both conventionally and genetically. A total of 108 mastitic cow milk samples were collected and sent to laboratory for microbiologic examination by veterinarians from 52 different private dairy farms in Balıkesir city which had 10 or more Holstein and/or Simmental cows, between November 2020 and June 2021. From the milk samples, 28 *Enterococcus* spp. isolates were identified. Vancomycin resistance was found in 13 of these isolates (46.4%) through disc diffusion and Vancomycin E-tests. The PCR method revealed the presence of the following resistance genes: *vanA* in 11 isolates, *vanB* in 4 isolates, *vanC1* in 1 isolate, *vanC2* in 10 isolates, and *vanC3* in 5 isolates. In 4 isolates, the *vanA*, *vanB*, *vanC2*, and *vanC3* genes were detected together. Additionally, 1 isolate contained *vanA*, *vanB*, and *vanC1* genes. Since *vanA* and *vanB* resistance genes can be transferred via transposons or plasmids, the presence of *vanA* and *vanB* positive *Enterococcus* spp. strains in this study suggests a potential risk for the spread of resistance genes, and poses a threat to public health. This study found a high prevalence of vancomycin resistance in enterococci isolated from mastitic milk, especially in terms of *vanA* and *vanB* genes positivity. There is a concern that milk intended for human consumption could become contaminated with *Enterococcus* spp., posing potential risks for human infections. Therefore, routine monitoring of VRE in milk is considered important for public health.

**Key words:** cow; enterococci; mastitis; PCR; vancomycin resistance

## Introduction

*Enterococcus* species, as part of the fecal flora, are commonly found in most mammals and birds (MELESE et al., 2020; ŞEKER et al., 2023). These important pathogens cause nosocomial and public health infections. In particular, *Enterococcus faecalis* and *Enterococcus faecium* are significant agents of bovine mastitis, (NAM et al., 2010; KEÇECİ et al., 2016; ROZANSKA et al., 2019; YANG et al., 2019; ŞEKER et al., 2023) a major disease affecting dairy cows and the dairy industry (ŞEKER et al., 2023). Previous studies have reported the incidence of *Enterococcus* mastitis in cows ranging from 0.3% to 60% (ŞEKER et al., 2023).

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Enterococci are part of the gastrointestinal flora in both humans and animals. However, *Enterococcus* spp., particularly *E. faecium*, has emerged as a significant nosocomial pathogen globally (ASLANTAŞ and TEK, 2019; DOLHAN and ERBAŞ, 2022). *Enterococcus* spp. are a public health problem due to their multidrug resistance (MDR) and their ability to horizontally transfer resistance genes to other bacteria, which contributes to increased morbidity and mortality (AHMED and BAPTİSTE, 2018; ÇETİNKAYA et al., 2020; ABDALİ et al., 2023; LYSİTSAS et al., 2023; PASCHOALİNİ et al., 2023).

Antibiotic-targeted treatments often lead to complex resistance mechanisms. Since enterococci exhibit multidrug resistance (MDR) to antibiotics such as aminoglycosides, ampicillin, and vancomycin, there are very few effective antibiotics available for treatment (ASLANTAŞ and TEK, 2019; YANG et al., 2019).

Additionally, previous research indicates that VRE strains carry antibiotic resistance genes that can be transmitted to humans through animals and animal-derived foods (EUCAST, 2017b; AQİB and ALSAYEQH, 2022; DE MORAES et al., 2023; ŞEKER et al., 2023). Enterococci are commonly found in foods, particularly in milk and dairy products. This prevalence can be attributed to the bacteria's ability to resist adverse environmental conditions (PASCHOALİNİ et al., 2023).

The effects of enterococci on public health highlight the need for a deeper understanding of this unique organism and its associated infections (EUCAST, 2017b).

Vancomycin acts by inhibiting the cell wall synthesis (PASCHOALİNİ et al., 2023). Since vancomycin is not commonly used for treating infections in animals, there are few reports of vancomycin-resistant *Staphylococcus aureus* (VRSA) strains in veterinary medicine (MOHAMADI et al., 2023).

Enterococci are Gram-positive, cocci-shaped, sporeless bacteria that are generally motile and catalase-negative. Key characteristics of enterococci include esculin hydrolysis and resistance to bile salts. The ability to resist bile salts helps enterococci to thrive, as bile salts inhibit the growth of many competing flora, particularly Gram-negative bacteria. Similarly, sodium azide acts as an inhibitor for Gram-negative bacteria (DOMIG et al., 2003).

Vancomycin resistance can be detected using minimal inhibitory concentration (MIC) testing and disc diffusion methods. It is crucial to incubate the plates for 24 hours to identify isolates with inducible resistance. The MIC test can be performed using agar dilution, broth microdilution, or gradient MIC testing methods (EZEH et al., 2023).

The European Committee of Antimicrobial Susceptability Testing (EUCAST) method for disc diffusion requires careful attention, including the examination of the interior of the zones for microcolonies. Clear and complete inhibition zones with diameters above the cutoff value are reported as susceptible to vancomycin (EUCAST, 2017b). In the MIC and gradient MIC methods, *Enterococcus* strains with MIC values greater than 4 are reported as resistant to vancomycin (EUCAST, 2017a; EUCAST, 2017b).

Disc diffusion tests are performed according to EUCAST guidelines. For some isolates with inducible resistance, a 24-hour incubation period is necessary to accurately detect resistance. Clear and complete inhibition zones with a diameter of ≥12 mm are reported as susceptible. Unclear or incomplete zones are reported as resistant, regardless of the zone diameter. (EUCAST, 2017a; EUCAST, 2017b).

Vancomycin resistance can be detected us- ing PCR targeting *vanA*, *vanB*, and *vanC* genes, along with other molecular techniques. (EUCAST, 2017b; ŞEKER et al., 2023).

Vancomycin resistance in *Enterococcus* spp. strains isolated from animals with mastitis in Turkey is typically determined phenotypically. This study is the first comprehensive investigation in Balıkesir, a leading city in milk production, to assess vancomycin resistance both phenotypically and genotypically. The research aimed to investigate the presence of *Enterococcus* spp. in mastit- ic cow milk and to detect vancomycin resistance along with the *vanA*, *vanB*, *vanC1*, *vanC2*, and *vanC3* genes in the isolates using both phenotypic and genotypic methods.

## Materials and methods

*Sample collection.* A total of 108 mastitic cow milk samples were collected and sent to laboratory for microbiologic examination by veterinarians from 52 different private dairy farms in Balıkesir city with had 10 or more Holstein and/or Simmental cows, between November 2020 and June 2021. These farms housed a total of 432 dairy cows. Approximately 5 ml of milk was collected into sterile containers after cleaning the teats with antiseptic, and the first milk was discarded. The samples were collected by veterinarians before administering any antibiotic treatment, and were analyzed in the laboratory for mastitis. Milk samples were taken from the udder lobes of cows exhibiting clinical mastitis symptoms, such as inflammation, pain and decreased milk yield, and also positive California Mastitis Test (CMT) results. These samples were delivered to the laboratory by veterinarians under cold chain conditions (2-8°C) and were promptly analyzed upon arrival. Samples that could not be analyzed immediately were stored at -20°C until analysis.

*Isolation and identification of Enterococci.* For the isolation and identification of enterococci, milk samples were first gently shaken to achieve homogenization and then inoculated onto 5% sheep blood agar (Merck, Germany), MacConkey agar (Merck, Germany), and Bile Aesculin Azide agar (Merck, Germany). The 5% sheep blood agar, Bile Aesculin Azide agar, and MacConkey agar plates were incubated at 37°C for 24 hours. (DOMIG et al., 2003; ŞEKER et al., 2023).

After incubation, macroscopic and microscopic evaluations were performed on the growth colonies. Gram staining, biochemical tests such as catalase and oxidase tests, and observation of growth characteristics on the agars were conducted to identify the isolates as *Enterococcus* spp. The isolates identified as *Enterococcus* spp. were then preserved in beads for further use (Cryobank, Mast Group, UK) at -20°C (METE and KALELİ, 2019; ŞEKER et al., 2023).

*Phenotypic determination and evaluation of vancomycin resistance.* Vancomycin resistance in *Enterococcus* spp. isolates was investigated and evaluated according to EUCAST standards using the disc diffusion and gradient MIC (E-test) methods on Mueller-Hinton agar (Merck, Germany). Gradient MIC (E-test) for vancomycin was conducted using Liofilchem MIC strips (Italy). For the disc diffusion test, 30 µg vancomycin discs (Oxoid, UK) were used (EUCAST, 2017b).

Evaluation and calculation of gradient MIC test were done according to EUCAST (EUCAST, 2017a; EUCAST, 2017b). In the gradient MIC test, *Enterococcus* spp. strains with MIC values great- er than 4 were reported as resistant to vancomycin (EUCAST, 2017a; EUCAST, 2017b).

In antibiogram and gradient MIC (E-test) tests, *Enterococcus faecalis* (ATCC® 29212™) strains were used as reference strains.

*Genotypic determination and evaluation of vancomycin resistance.* All *Enterococcus* spp. isolates were inoculated into Brain Heart Infusion Broth (BHI, Oxoid, UK) and incubated at 37°C for 24 hours to obtain pure cultures. After incubation, 1 mL of the BHI broth culture was centrifuged at 5000×g for 10 minutes. The supernatant was then removed, and DNA was extracted from the pellet using the GeneJET Genomic DNA Purification Kit (Thermo Scientific, USA) and the DNA Purification Protocol for Gram-positive bacteria based on spin column technology. *vanA*, *vanB*, *vanC* (*C1*, *C2* and *C3*) genes were analyzed by PCR using that genes specific primers, which were used by researchers in previous studies (FRAIMOW et al., 1994; KLARE et al., 1995; SATAKE et al., 1997; CLARK et al., 1998; LEMCKE and BÜLTE, 2000) (Table 1).

The PCR for the *vanA* gene was performed using a two-step protocol based on the methods of KLARE et al. (1995) and LEMCKE and BÜLTE (2000). The PCR reaction mixture for the *vanA* gene was prepared in a total volume of 25 µL, containing 2 µL of DNA extract, using the DreamTaq PCR Master Mix (2X) Kit (Thermo Scientific, USA). Amplification conditions were set according to the protocols described by KLARE et al. (1995) and LEMCKE and BÜLTE (2000).

PCR for the *vanB* gene was performed following the protocols of FRAIMOW et al. (1994) and LEMCKE and BÜLTE (2000). The PCR reaction mixture, with a total volume of 25 µL, contained

Table 1. vanA, vanB, vanC (C1, C2 and C3) gene specific primer sequences, target genes, base pairs and references

| Primers | Sequences | Target genes | Base Pairs | References |
|---|---|---|---|---|
| **vanA I**<br>**vanA II** | TCT gCAATA gAg ATA gCC gC<br>GG AgT AgC TAT CCC AgC ATT | *VanA* | 377 bp | Lemcke and Bülte (2000)<br>Klare et al. (1995) |
| **vanB I**<br>**vanB II** | gCT CCg CAg CCT gCA Tgg ACA<br>ACg ATg CCg CCA TCC TCC TgC | *VanB* | 529 bp | Lemcke and Bülte (2000)<br>Fraimow et al. (1994) |
| **vanC1 I**<br>**vanC1 II** | gAAAgA CAA CAg gAA gAC CgC<br>TCg CAT CAC AAg CAC CAA TC | *VanC1* | 796 bp | Lemcke and Bülte (2000)<br>Clark et al. (1998) |
| **vanC2 I**<br>**vanC2 II** | Cgg ggA AgA Tgg CAg TAT<br>CgC Agg gAC ggT gAT TTT | *VanC2* | 484 bp | Lemcke and Bülte (2000)<br>Satake et al. (1997) |
| **vanC3 I**<br>**vanC3 II** | gCC TTT ACT TAT TgT TCC<br>gCT TgT TCT TTg ACC TTA | *VanC3* | 224 bp | Lemcke and Bülte (2000)<br>Clark et al. (1998) |

2 µL of DNA extract and was prepared using the DreamTaq PCR Master Mix (2X) Kit (Thermo Scientific, USA). (FRAIMOW et al., 1994; LEMCKE and BÜLTE, 2000).

PCR and amplification conditions for all *vanC* genes (*C1*, *C2*, and *C3*) were carried out according to the methods described by SATAKE et al. (1997), CLARK et al. (1998), and LEMCKE and BÜLTE (2000). The PCR reaction mixture, with a total volume of 25 µL, contained 2 µL of DNA extract and was prepared using the DreamTaq PCR Master Mix (2X) Kit (Thermo Scientific, USA) (CLARK et al., 1998; YANG et al., 2019; LEMCKE and BÜLTE, 2000).

All PCR amplicons were electrophoresed at 180 V for 30 minutes on a 1.5% agarose gel (Prona, USA) stained with BlueJuice dye (Thermo Scientific, USA) and using a DNA molecular weight marker (Gene Ruler 100 bp DNA Ladder Plus, Thermo Scientific, USA). The results were visualized using a gel imaging system (EBOX CX5 TS EDGE, Vilber).

In PCR tests, *Enterococcus faecalis* (ATCC® 29212™) was used as the reference strain, and a PCR mix without DNA was used as the negative control.

**Results**

After incubation, suspicious *Enterococcus* spp. colonies growing on 5% sheep blood agar (Merck, Germany), MacConkey agar (Merck, Germany), and Bile Aesculin Azide agar (Merck, Germany) were selected and purified. Following purification, Gram staining was performed, and Gram-positive cocci strains were identified through biochemical tests.

After evaluating macroscopic and microscopic morphologies, biochemical tests, and growth characteristics on various agars, a total of 28 *Enterococcus* spp. (25.92%) were isolated from 108 mastitic cow milk samples (Fig. 1).

Phenotypic vancomycin resistance was calculated and evaluated according to EUCAST (2017a) and EUCAST (2017b). On the gradient MIC test, *Enterococcus* spp. strains with MIC values greater than 4 (>4) were recorded as vancomycin resistant. So, 13 (46.4%) enterococci isolates out of 28 enterococci isolates were detected to be resistant to vancomycin with vancomycin gradient MIC (E-tests) and disc diffusion tests (Fig. 2, Fig. 3).

Vancomycin resistance genes in *Enterococcus* spp. isolates were investigated using PCR, confirming their presence. All investigated vancomycin resistance genes were identified genotypically.

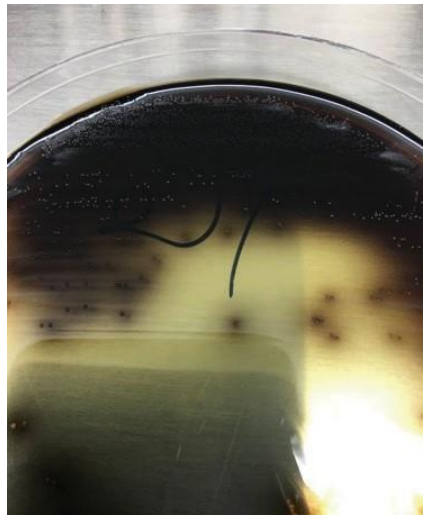

Fig. 1. Isolated *Enterococcus* spp. on Bile aesculin azide agar

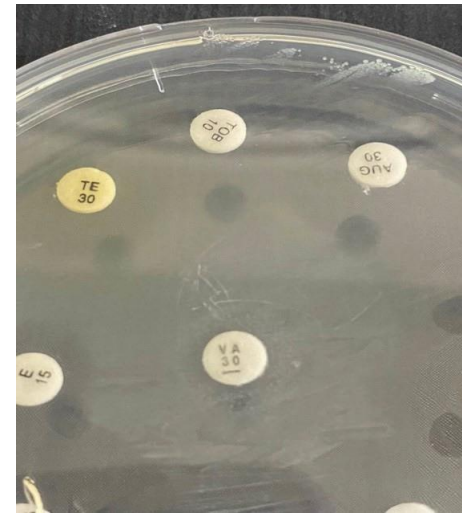


Fig. 2. Disc diffusion test vancomycin resistance results of *Enterococcus* spp.

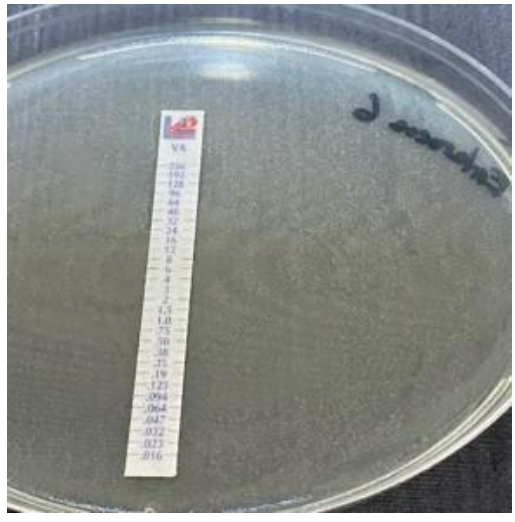

Fig. 3. Gradient MIC test vancomycin resistance results of *Enterococcus* spp.

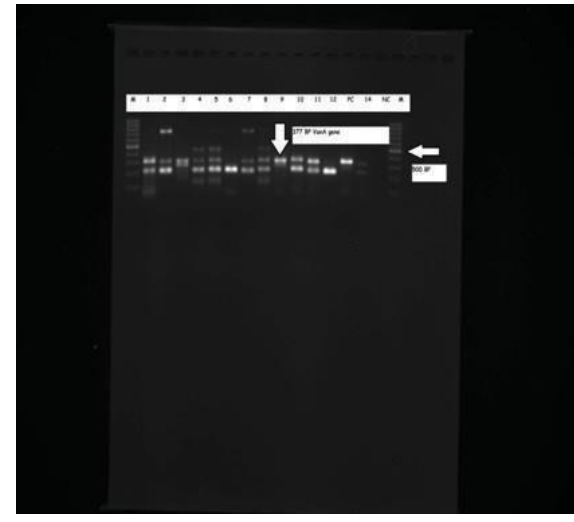

Fig. 4. PCR results of *vanA* gene for *Enterococcus* spp. isolates

(M: Marker, PC: Positive control, NC: Negative control, Line 9: positive isolate)

By PCR, the following vancomycin resistance genes were detected: *vanA* in 11 isolates, *vanB* in 4 isolates, *vanC1* in 1 isolate, *vanC2* in 10 isolates, and *vanC3* in 5 isolates (Fig. 4, Fig. 5, Fig. 6).

In this study, the *vanA* gene was the most frequently detected vancomycin resistance gene by PCR, followed by *vanC2* as the second most prevalent.

*vanA, vanB, vanC2* and *vanC3* genes were detected together in 4 isolates.

While, *vanA, vanB* and *vanC1* genes were detected together in 1 isolate.

The PCR results for vancomycin resistance genes in enterococci are presented in Table 2.

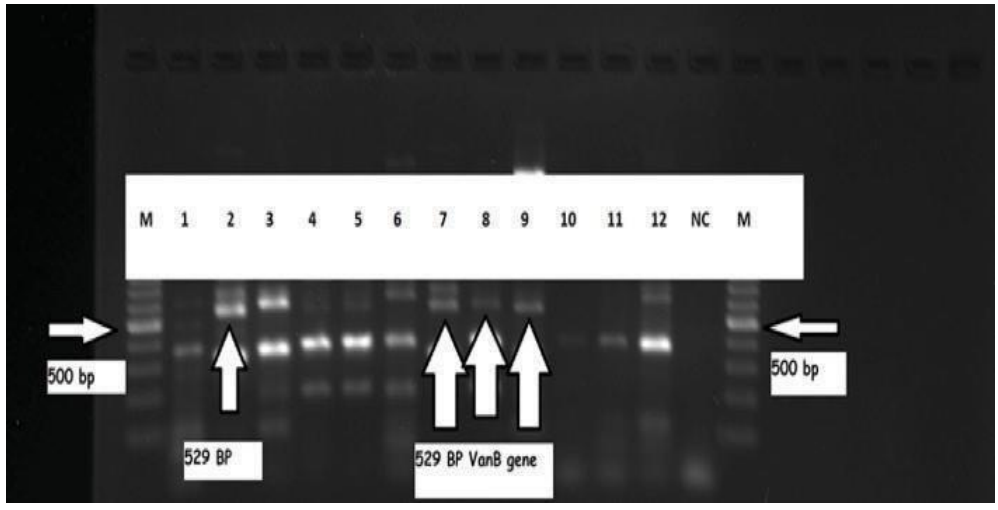


Fig. 5. PCR results of *vanB* gene for *Enterococcus* spp. isolates
(M: Marker, PC: Positive control, NC: Negative control, Line 2, 7-9: positive isolates)

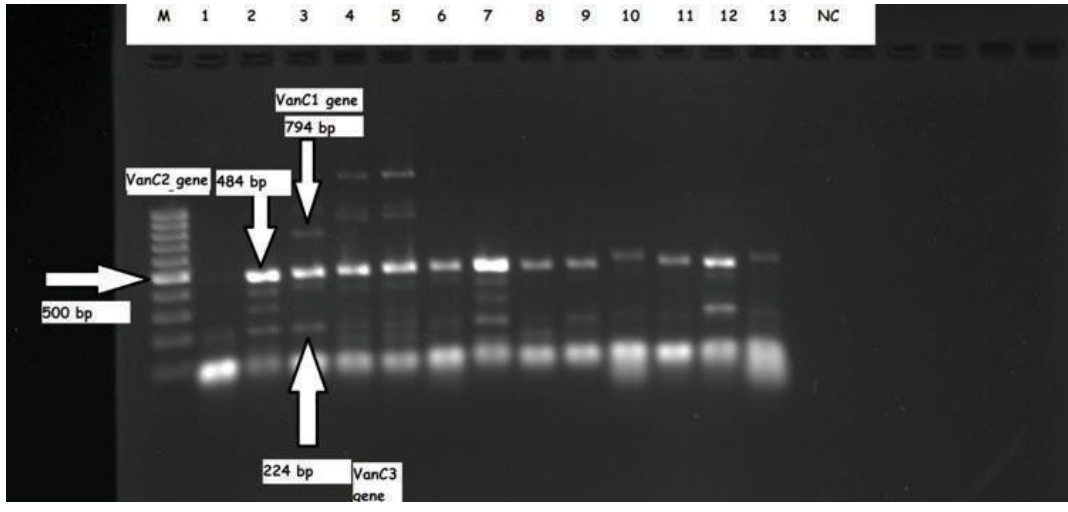


Fig. 6. PCR results of *vanC* genes for *Enterococcus* spp. isolates
(M: Marker, PC: Positive control, NC: Negative control, Line 2: *vanC2* gene positive isolate, Line 3: *vanC1* and *vanC3* genes positive isolate)

Table 2. PCR results of vanA, vanB, vanC (C1, C2 and C3) genes

| Vancomycin resistance genes | Detected enterocci number (n) |
|---|---|
| *vanA* | 11 |
| *vanB* | 4 |
| *vanC1* | 1 |
| *vanC2* | 10 |
| *vanC3* | 5 |
| *vanA+vanB+vanC1* | 1 |
| *vanA+vanB+vanC2+vanC3* | 4 |

**Discussion**

This research investigated the presence of *Enterococcus* spp. and VRE strains in mastitic milk samples from cows using gradient MIC (E-Test) and PCR for the first time in Balıkesir province, which is the leading city in milk production in Türkiye.

While *Enterococcus* spp. was not previously considered a major pathogen in the etiology of cow mastitis, recent years have seen an increased recognition of these bacteria as significant contributors to mastitis (ERBAŞ et al., 2016; ŞEKER et al., 2023).

In Brazil, DE MORAES et al. (2023) stated that VRE was not detected in 53 intermediate resistant *Enterococcus* spp. strains isolated from cows with clinical mastitis and from bulk milk tanks.

ABDALİ et al. (2023) stated that they had isolated 61 (20.3%) enterococci from mastitic cow milk samples. They also reported 40% and 30% vancomycin-resistant strains for *E. faecalis* and *E. faecium*, respectively.

In Turkey, ERBAŞ et al. (2016) found that the *vanA* gene was detected in one (1.8%) of 56 *E. faecalis* strains isolated from bovine mastitis. They also stated that no vancomycin resistance was found in *Enterococcus* spp. isolated in their study.

In another study conducted in Turkey, KEÇECİ et al. (2016) reported finding *vanB* gene positivity in 11 of 57 *E. faecalis* strains (19%), and in 7 of 8 E. faecium strains (88%). Additionally, they detected a combination of *vanC2/C3* genes in one *E. faecium* strain and both *vanB* and *vanC2/C3* genes in two E. faecium strains.

KOLDAŞ ÜRER et al. (2022), in their study on buffalo milk from Çorum province, found *Enterococcus* spp. in 65 of 200 mastitic milk samples (32.5%). They isolated these strains but did not detect vancomycin resistance in any of the isolates.

In a study published last year in Turkey, ŞEKER et al. (2023) revealed that vancomycin resistance genes were identified in 71 *Enterococcus* spp. strains, with 19 (26.7%) of these strains being diagnosed as VRE. They found that 5 strains (7%) carried the *vanA* gene, 10 strains (14%) had the *vanB* gene, and 12 strains (16.9%) contained the *vanC2/C3* genes. Notably, no *vanC1* gene was detected in any of the strains. Additionally, they reported that *vanA* and *vanB* gene positivity was observed exclusively in *E. faecalis* and *E. faecium* strains, whereas *vanC2/C3* genes were present in all VRE strains.

The 46.4% VRE isolation rate obtained in this study (13 of 28 *Enterococcus* spp. strains) was significantly higher than the rates reported in previous studies.

Previous studies indicate that foods of animal origin may be a source of contamination for VRE infections in humans, with VRE strains carrying antibiotic resistance genes potentially playing a significant role in the spread of vancomycin resistance. In this research, *Enterococcus* spp. isolated from mastitic cow milk samples were found to be of considerable epidemiological and public health importance. While vancomycin resistance has been commonly assessed phenotypically in previ- ous research, there have been relatively few stud- ies focusing on genotypic detection (ERBAŞ et al., 2016; GÜNGÖR et al., 2023; ŞEKER et al., 2023).

ŞEKER et al. (2023) found that the detection of *vanA* and *vanB* gene positivity in E. faecium and E. faecalis strains was consistent with the established knowledge that *vanA* and *vanB* resistance genes are more commonly encountered clinically. Consistent with the findings of ŞEKER et al. (2023) and the general consensus, this study also identified *vanA*, *vanC2*, and *vanB* genes as the most prevalent vancomycin resistance genes in the isolated VRE strains.

MOHAMADI et al. (2023) declarated that VRE strains were isolated from various food samples including animal-origin foods in Iran. They also noted that *vanA*, *vanB* and *vanC* genes were the most commonly detected genes in VRE strains in Iran.

KALATEH RAHMANİ et. al. (2022) report- ed that bovine vancomycin resistant enterococci (VRE) isolates can be the source of vancomycin resistance for human enterococci.

In this study, all the vancomycin resistance genes (*vanA, vanB, vanC1, vanC2* and *vanC3*) were detected in isolated *Enterococcus* spp. strains. The *vanA* gene was the most detected vancomycin resistance gene by PCR, followed by *vanC2* as the

second most prevalent. The *vanB* gene was detected in 4 *Enterococcus* spp. strains.

In this research, *VanA*, *VanB* and *VanC2* and *VanC3* genes were found together in 4 isolates, while *VanA*, *VanB* and *VanC1* genes were detected together in 1 isolate.

Since *vanA* and *vanB* resistance genes can be transferred via transposons or plasmids (NİLLSON, 2012; LYSİTSAS et al., 2023), the *vanA* and *vanB* positive *Enterococcus* spp. strains identified in this study may contribute to the spread of resistance genes and, consequently, pose a potential risk to public health.

*VanA*-producing strains exhibit resistance to both vancomycin and teicoplanin, whereas *VanB*-producing strains usually remain suscepti- ble to teicoplanin due to the lack of induction of the resistance operon (EUCAST, 2017b). In this study, *Enterococcus* spp. isolates were found to be positive for *vanA* and *vanB* genes by PCR, with some isolates being positive for both *vanA* and *vanB* genes. From a public health perspective, it is considered that treatment difficulties may arise in human infections that could originate from cow's milk.

EUCAST (2017b) has reported that the MIC values of vancomycin for *Enterococcus* spp. isolates with the *vanA* gene range between 64-1024 and for isolates with the *vanB* gene between 4-1024. In this study, the high MIC values obtained for *Enterococcus* spp. isolates positive for both *vanA* and *vanB* genes are consistent with the EUCAST (2017b) report.

Recent research has proposed various alternatives to antibiotic treatment for mastitis in cows, including herbal remedies, essential oils, nanotechnology, and polymers. These alternative treatments are non-invasive and do not contribute to resistance. Researchers have emphasized that these treatment protocols should be precise, easy to administer, cost-effective, effective, reliable, and well-documented (MORALES-UBALDO et al., 2023).

In conclusion, this study represents the first comprehensive phenotypic and genotypic investigation of *Enterococcus* spp. and VRE strains isolated from mastitic milk samples in Balıkesir province, Turkey. Our findings indicate that enterococci are a significant cause of mastitis in dairy cows in Balıkesir. Therefore, it is essential that milk samples be examined not only for specific mastitis pathogens but also for *Enterococcus* species in routine diagnostic procedures. Given the risk of vancomycin resistance gene transfer to humans, particularly through animal-based foods, and the potential for contamination from inadequately heat-treated dairy products, it is crucial to address these concerns to prevent the spread of resistance.

In this research, due to the high prevalence of VRE detected in the milk of cows with mastitis, it is anticipated that this rate may increase in the future. Consequently, there is concern that milk intended for human consumption could become contaminated with *Enterococcus* spp., posing potential risks for human infections. Therefore, routine monitoring of VRE in milk is considered important for public health.

As a result, it was determined that vancomy- cin resistance was both common and high in enterococci isolated from milk with mastitis, especially in terms of *vanA* and *vanB* gene positivity. It is suggested that future studies should focus on the spread of vancomycin-resistant *Enterococcus* (VRE) infections and the continuous monitoring of VRE in farm animals.

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**Author contribution statement**

Orkun Babacan conceived and planned the experiments, carried out the experiments, contributed to sample preparation, contributed to the interpretation of the results, took the lead in writing the manuscript, provided critical feedback and helped shape the research, analysis and manuscript.

**Declaration of competing interest**

The authors declared that there is no conflict of interest.

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This study was presented as oral presentation in 6th International Congress on Veterinary and Animal Sciences (ICVAS) in 2-4 September 2021, Kırıkkale province, Turkey, Online Congress.

**Data availability statement**

The data supporting this study's findings are available from the corresponding author upon reasonable request.

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**BABACAN, O.: Detekcija gena rezistencije na vankomicin primjenom lančane reakcije polimerazom u uzorcima enterokoka izoliranih iz mlijeka krava s mastitisom. Vet. arhiv 95, 381-391, 2025.**

**SAŽETAK**

Cilj rada bio je istražiti prevalenciju enterokoka i prisutnost gena rezistencije na vankomicin u kontekstu mastitisa koji uzrokuju ekonomske gubitke i smanjen prinos u mliječnih krava u pokrajini Balıkesir. Ukupno je prikupljeno 108 uzoraka mlijeka od krava s mastitisom i poslano na mikrobiološki pregled od strane veterinara sa 52 različite privatne farmi mliječnih krava u gradu Balıkesir, koje su imale 10 ili više Holstein i/ili Simmental krava, u razdoblju od studenog 2020. do lipnja 2021. godine. Iz uzoraka mlijeka identificirano je 28 izolata bakterije *Enterococcus* spp. Primjenom disk-difuzijskog i Vancomycin E-testa, rezistencija na vankomicin pronađena je u 13 izolata (46,4%). Metodom PCR ustanovljena je prisutnost sljedećih gena rezistencije: *vanA* u 11 izolata, *vanB* u 4 izolata, *vanC1* u 1 izolatu, *vanC2* u 10 izolata i *vanC3* u 5 izolata. U 4 izolata pronađeni su zajedno geni *vanA*, *vanB*, *vanC2* i *vanC3*. Također, jedan je izolat sadržavao gene *vanA*, *vanB* i *vanC1*. Budući da se geni rezistencije *vanA* i *vanB* mogu prenijeti transposomima i plazmidima, prisutnost sojeva enterokoka pozitivnih na *vanA* i *vanB* u ovom istraživanju upućuje na potencijalni rizik za širenje gena rezistencije što predstavlja prijetnju javnom zdravstvu. Istraživanjem je otkrivena visoka prevalencija rezistencije na vankomicin kod enterokoka izoliranih iz uzoraka mlijeka krava s mastitisom, posebno s obzirom na pozitivnost nalaza na gene *vanA* i *vanB*. Postoji zabrinutost da bi mlijeko namijenjeno za ljudsku prehranu moglo biti kontaminirano enterokokima, čime se povećava potencijalni rizik za infekciju ljudi. Stoga bi rutinsko praćenje vankomicin rezistentnih enterokoka (VRE) u mlijeku bilo iznimno važno za javno zdravstvo.

**Ključne riječi:** krava; enterokok; mastitis; PCR; rezistencija na vankomicin