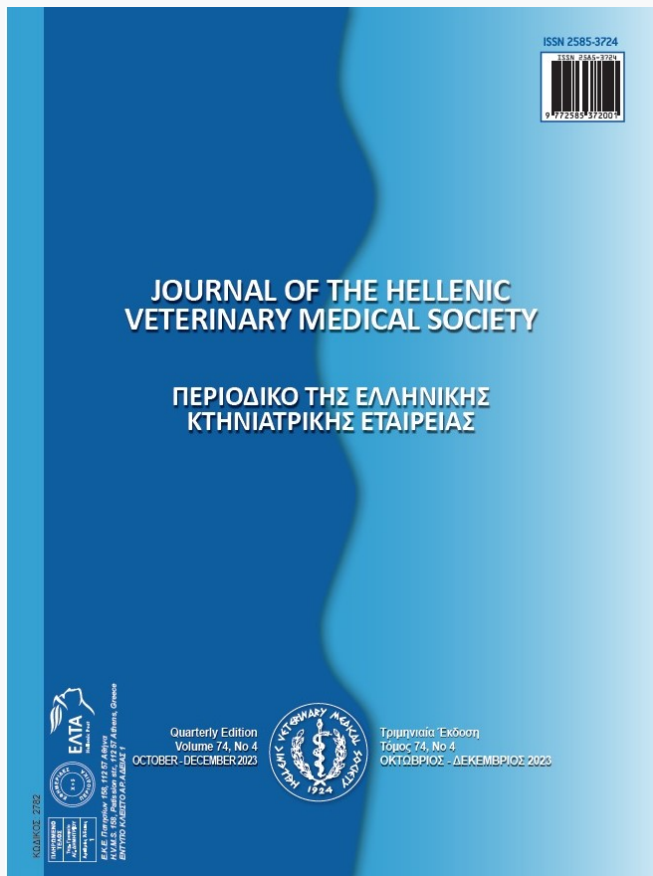


## Journal of the Hellenic Veterinary Medical Society

Vol 74, No 4 (2023)



### Presence of genes encoding aminoglycoside-modifying enzyme (AME) and virulence factors in high-level aminoglycoside-resistant (HLAR) Enterococcus strains isolated from retail chicken meat in Turkey

*M Yalçın, B Özden Tuncer, D Akpınar Kankaya, Y Tuncer*

doi: [10.12681/jhvms.30850](https://doi.org/10.12681/jhvms.30850)

Copyright © 2024, M Yalçın, B Özden Tuncer, D Akpınar Kankaya, Y Tuncer



This work is licensed under a [Creative Commons Attribution-NonCommercial 4.0](https://creativecommons.org/licenses/by-nc/4.0/).

#### To cite this article:

Yalçın, M., Özden Tuncer, B., Akpınar Kankaya, D., & Tuncer, Y. (2024). Presence of genes encoding aminoglycoside-modifying enzyme (AME) and virulence factors in high-level aminoglycoside-resistant (HLAR) Enterococcus strains isolated from retail chicken meat in Turkey. *Journal of the Hellenic Veterinary Medical Society*, 74(4), 6441–6450. <https://doi.org/10.12681/jhvms.30850>

## Presence of genes encoding aminoglycoside-modifying enzyme (AME) and virulence factors in high-level aminoglycoside-resistant (HLAR) *Enterococcus* strains isolated from retail chicken meat in Türkiye

M. Yalçın<sup>1</sup> , B. Özden Tuncer<sup>1</sup> , D. Akpınar Kankaya<sup>2</sup> , Y. Tuncer<sup>1,\*</sup> 

<sup>1</sup>Süleyman Demirel University, Faculty of Engineering and Natural Sciences, Department of Food Engineering, Isparta, Türkiye

<sup>2</sup>Department of Food Technology, Gelendost Vocational School, Isparta University of Applied Sciences, Isparta, Türkiye

**ABSTRACT:** In this study, the presence of aminoglycoside-modifying enzyme (AME) and virulence factor genes were investigated in previously isolated 32 high-level aminoglycoside-resistant (HLAR) *Enterococcus* strains isolated from retail chicken meat in Türkiye. At least one AME-encoding gene was detected in HLAR enterococci by polymerase chain reaction (PCR). The *ant(6')-Ia* was identified as the most prevalent (87.5%, 28/32) AME gene. The *aph(3')-IIIa* (78.13%, 25/32), *ant(4')-Ia* (68.75%, 22/32), *aph(2'')-Ib* (62.5%, 20/32), *aac(6')-Ie-aph(2'')-Ia* (21.88%, 7/32) and *aph(2'')-Ic* (9.38%, 3/32) are the other detected AME-encoding genes in strains. The *aph(2'')-Id* was found in none of the HLAR strains. The *aph(2'')-Ib* and *ant(6')-Ia* were identified as the most frequently AME-encoding genes in high-level gentamicin-resistant (HLGR) and high-level streptomycin-resistant (HLSR) strains, respectively. All HLAR strains showed  $\alpha$ -hemolytic activity except *E. durans* MG13.4 and *E. casseliflavus* MGM111.1, which were exhibited  $\beta$ - and  $\gamma$ -hemolytic activity, respectively. Among the 32 HLAR strains, only *E. faecalis* MSE61.1 and *E. avium* MSE63.1 were found capable of hydrolyzing gelatine. It was determined that all HLAR strains, except *E. durans* MGE13.1 and MGE63.1, contain at least one virulence factor gene. The *efaA<sub>fm</sub>* (87.5%, 28/32), *acm* (65.63%, 21/32) and *gelE* (37.5%, 12/32) were found to be the most prevalent virulence factor genes. HLAR enterococci strains that have the virulence factor genes may pose a risk to consumer health.

**Keywords:** Chicken meat; *Enterococcus*; high-level aminoglycoside resistant; aminoglycoside-modifying enzyme; virulence factor gene

*Corresponding Author:*

Yasin Tuncer, Süleyman Demirel University, Faculty of Engineering and Natural Sciences, Department of Food Engineering, 32260, Isparta, Türkiye.  
E-mail address: yasintuncer@sdu.edu.tr

*Date of initial submission:* 18-07-2022  
*Date of acceptance:* 10-12-2022

## INTRODUCTION

Enterococci are lactic acid bacteria that are present in the natural microflora of the human and animal gastrointestinal tracts (Guzman Prieto et al., 2016). In addition, they have been used as starter or adjunct cultures for centuries in the production of various fermented foods (Hugas et al., 2003; M'hir et al., 2012). However, they have also been known as important nosocomial pathogens since the 1970s, are multi-drug resistant, and have a wide range of virulence factors (Arias and Murray, 2012). Enterococci show natural resistance to many antibiotics, as well as easily gain resistance to clinically important different groups of antibiotics, increasing the pathogenicity of these bacteria (Yoğurtçu and Tuncer, 2013; Abaueknaga et al., 2016). As a result of the inability of aminoglycosides to penetrate the cell wall of enterococci, these bacteria have a moderate intrinsic low-level resistance to them (Hollenbeck and Rice, 2012). In clinical practice, aminoglycoside antibiotics such as gentamicin and streptomycin are frequently utilized (Özdemir and Tuncer, 2020). In recent years, it has been reported that high-level of gentamicin (HLGR) ( $MIC \geq 500 \mu\text{g/mL}$ ) and streptomycin-resistant (HLSR) ( $MIC \geq 2000 \mu\text{g/mL}$ ) enterococci have been isolated from clinical samples (Niu et al., 2016), ready-to-eat meat samples (Chajęcka-Wierzchowska et al., 2016), raw milk (Özdemir and Tuncer, 2020; Kang et al., 2021), dairy products (Chajęcka-Wierzchowska et al., 2020; Özdemir and Tuncer, 2020) and retail chicken meat (Choi and Woo, 2013; Kim et al., 2018; Kim et al., 2019; Yalçın and Tuncer, 2021). In enterococci, gains of genes that code for AMEs such as phosphotransferases, acetyltransferases, and nucleotidyltransferases result in high-level acquired resistance to aminoglycosides (Guzman Prieto et al., 2016). AAC(6')-APH(2''), a bifunctional AME encoded by the *aac(6')-Ie-aph(2'')-Ia* gene, mediates high-level gentamicin resistance. The monofunctional AME encoding genes *aph(2'')-Ib*, *aph(2'')-Ic* and *aph(2'')-Id* are also responsible for high-level gentamicin resistance. The *ant(6')-Ia* gene encoding Ant(6')-Ia, an adenylyltransferase, is responsible for high-level streptomycin resistance (Hollenbeck and Rice, 2012). Other monofunctional AME-encoding genes found in enterococci include *aph(3')-IIIa* and *ant(4')-Ia*. The *aph(3')-IIIa* gene encodes an aminoglycoside phosphotransferase, Aph(3')-IIIa, which confers resistance to kanamycin and neomycin, whereas the *ant(4')-Ia* gene encodes a nucleotidyltransferase, Ant(4'')-Ia, which confers resistance to tobramycin,

amikacin, neomycin, and kanamycin (Hauschild et al., 2008; Hollenbeck and Rice, 2012).

The cause of infections due to enterococci is the presence of surface factors that affect the colonisation of host cells and secretory agents that damage the tissues. Virulence factors have a role in the pathogenesis of enterococcal infections by mediating adhesion, colonization, and invasion into host tissues, as well as modulating host immunity, which increase the infection's severity (Sava et al., 2010; Chajęcka-Wierzchowska et al., 2017). The main virulence factors identified in enterococci species include i) surface factors; aggregation protein (*agg*), collagen-binding protein (*ace*, *acm*), cell wall adhesins (*efaA<sub>fm</sub>*, *efaA<sub>fs</sub>*), extracellular surface protein (*esp<sub>fm</sub>*, *esp<sub>fs</sub>*), ii) secretory agents; cytolysin (*cylM*, *cylB*, *cylA*), gelatinase (*gelE*), hyaluronidase (*hyl*) and iii) sex pheromones (*cpd*, *cob*, *ccf*, *cad*) (Chajęcka-Wierzchowska et al., 2017; Akpınar and Tuncer, 2022).

There is limited information on the prevalence of AME and virulence factor genes in HLAR *Enterococcus* strains isolated from retail chicken meat in Türkiye. Therefore, this study aimed to investigate the genes encoding AMEs (*aac(6')-Ie-aph(2'')-Ia*, *aph(2'')-Ib*, *aph(2'')-Ic*, *aph(2'')-Id*, *ant(4')-Ia*, *ant(6')-I*, *aph(3')-IIIa*) and virulence factors (*agg*, *efaA<sub>fm</sub>*, *efaA<sub>fs</sub>*, *esp<sub>fm</sub>*, *esp<sub>fs</sub>*, *ace*, *acm*, *cylM*, *cylB*, *cylA*, *gelE*, *hyl*, *cpd*, *cob*, *ccf*, *cad*) in HLAR *Enterococcus* strains isolated from retail chicken meat in Türkiye.

## MATERIAL AND METHODS

### HLAR *Enterococcus* strains

A total of 32 HLAR *Enterococcus* strains previously isolated from 112 retail chicken meat samples (39 chicken wings, 37 chicken breasts, 20 chicken legs, 16 chicken drumsticks) were used in this study. According to MIC test results for gentamicin and streptomycin, one, 13 and 18 of 32 HLAR *Enterococcus* strains were found to be HLGR, HLSR and both HLGR and HLSR, respectively. The 32 HLAR isolates were identified as *Enterococcus* species by conventional tests and genus-specific polymerase chain reaction (PCR). The identification of the 18 *E. faecium*, five *E. faecalis*, five *E. durans*, and one *E. casseliflavus* strains were done species-specific by PCR while three *E. avium* strains were identified by 16S rDNA sequence analysis (Yalçın and Tuncer, 2021). Stock cultures of 32 HLAR strains were stored in antibiotic-containing de man Rogosa and Sharpe (MRS) broth with the addition of sterile glycerol at -32 °C.

### Genomic DNA extraction

Genomic DNA from HLAR strains was extracted according to the method of Cancilla et al. (1992). The 500 µL of overnight cultures of HLAR isolates were centrifuged at 15.493 x g for 5 minutes in 2 mL microcentrifuge tubes. The pellets were resuspended in the same volume of lysis buffer and incubated at 37 °C for 30 minutes. Then 30 µL of SDS (10%, w/v) was added to the tubes and incubated at 80 °C for 5 minutes. After incubation, 700 µL of phenol: chloroform (1:10, v/v) was added to the tubes and centrifuged at 15.493 x g for 5 minutes. The upper phase was taken by micropipette and transferred to new tube. The 700 µL of propan-2-ol (Merck, Darmstadt, Germany) was added to the tubes for the precipitation of nucleic acids. Nucleic acids were pelleted by centrifugation at 15.493 x g for 5 minutes and dissolved in 50 µL of Tris-EDTA buffer (pH 8.0).

### Detection of AME-encoding genes

Detection of AME-encoding genes in HLAR *Enterococcus* was done using specific primers by PCR according to Vakulenko et al. (2003) and Niu et al. (2016). AME genes, primers, product sizes and PCR protocols are given in Table 1. PCR was performed in 50 µL PCR mixture prepared by adding 25 µL of PCR master mix (Thermo Scientific, USA), 20 µL nuclease-free water, 3 µL of template DNA and 1 µL of each primer to 0.2 mL of PCR tube (Thermo Scientific). PCR was carried out in gradient thermal cycler (TurboCycler 2 Blue-Ray Biotech Ltd., Taiwan). The agarose gel electrophoresis of amplified products was done on agarose gels (2%, w/v) in Tris-acetate-EDTA buffer at 85 V for 90 minutes. The gels were stained with ethidium bromide (0.2 µg/mL), visualized on a UV transilluminator (Vilber Lourmat, France), and photographed via a digital camera (D5100 Nikon Inc., Japan). The aminoglycoside-resistant strains *E. faecalis* ATCC 51299 (*aac(6')-Ie-aph(2'')-Ia*<sup>+</sup>, *ant(6')-Ia*<sup>+</sup>, *aph(3')-IIIa*<sup>+</sup>) and *E. faecium* ATCC 51599 (*ant(6')-Ia*<sup>+</sup>, *aph(3')-IIIa*<sup>+</sup>) were used as positive controls. The aminoglycoside-susceptible strain *E. faecalis* ATCC 29212 was used as a negative control.

### Hemolytic and gelatinase activities

Hemolytic activity of HLAR *Enterococcus* strains was detected on sheep blood agar (Liofilchem, Rose-to degli Abruzzi, Italy). Petri dishes were incubated at 37 °C for 48 hours. The hemolytic reaction was evaluated as β (clear zone formation around the colony), α (fuzzy greenish zone formation) or γ (non-zone

formation) hemolytic activity (Cariolato et al., 2008). β-hemolytic *S. aureus* ATCC 25923 was used as a control strain.

HLAR *Enterococcus* strains were cultured overnight and then transferred on Todd-Hewitt agar medium (Liofilchem) containing 3% gelatine (Merck) and incubated at 37 °C for 24 hours. After, Petri dishes were kept refrigerated at 4 °C for 5 hours. The presence of opaque zones surrounding the colonies was evaluated as a positive result (Eaton and Gasson, 2001). Gelatinase positive *E. faecalis* NYE7 was used as a control strain (Inoğlu and Tuncer, 2013).

### Detection of virulence factor genes

The virulence factor genes encoding aggregation protein (*agg*), cell wall adhesins (*efaA<sub>fm</sub>*, *efaA<sub>fs</sub>*), cell wall-associated protein (*esp<sub>fm</sub>*, *esp<sub>fs</sub>*), collagen-binding protein (*ace*, *acm*), cytolysin (*cylM*, *cylB*, *cylA*), gelatinase (*gelE*), hyaluronidase (*hyl*) and sex pheromones (*cpd*, *cob*, *ccf*, *cad*) were investigated in HLAR *Enterococcus* strains by PCR (Eaton and Gasson, 2001; Vankerckhoven et al., 2004; Reviriego et al., 2005; Camargo et al., 2006; Ben Belgacem et al., 2010). Virulence factor genes, primers, product sizes and PCR protocols are given in Table 1. PCR was performed in 50 µL reaction mixtures prepared as described above. The PCR products were verified on 2% (w/v) agarose gel and stained as described above. The *E. faecalis* ATCC 29212 (*agg*<sup>+</sup>, *efaA<sub>fm</sub>*<sup>+</sup>, *efaA<sub>fs</sub>*<sup>+</sup>, *esp<sub>fm</sub>*<sup>+</sup>, *esp<sub>fs</sub>*<sup>+</sup>, *ace*<sup>+</sup>, *acm*<sup>+</sup>, *cylM*<sup>+</sup>, *cylB*<sup>+</sup>, *cylA*<sup>+</sup>, *gelE*<sup>+</sup>, *hyl*<sup>+</sup>, *cpd*<sup>+</sup>, *cob*<sup>+</sup>, *ccf*<sup>+</sup>, *cad*<sup>+</sup>) was used as a positive control strain.

## RESULTS

### Detection of AME-encoding genes

In this study, it has been determined that HLAR *Enterococcus* strains contain between one and five AME-encoding genes (Table 2). The most prevalent AME-encoding genes in HLAR *Enterococcus* strains was found to be *aph(3')-IIIa* (78.13%, 25/32) and *ant(6')-Ia* (75.00%, 24/32) (Figure 1). These genes were followed by *ant(4')-Ia* (68.75%, 22/32), *aph(2'')-Ib* (62.50%, 20/32), *aac(6')-Ie-aph(2'')-Ia* (21.88%, 7/32) and *aph(2'')-Ic* (9.38%, 3/32). However, *aph(2'')-Id* gene was not detected in any of the HLAR strains. The distribution of AME-encoding genes in HLAR *Enterococcus* strains is given in Table 3. Twelve distinct AME-encoding gene pattern types were discovered in HLAR *Enterococcus* strains using PCR amplification products. The most common



AME-encoding gene pattern type of HLAR *Enterococcus* strains was type I (34.38%, 11/32) included *aph(3')-IIIa*, *ant(4')-Ia*, *ant(6')-Ia*, and *aph(2'')-Ib* genes.

### Hemolytic and gelatinase activities

The hemolytic and gelatinase activities of strains were phenotypically tested. The results of the hemolytic activity test revealed that the majority (93.75%, 29/31) of the HLAR *Enterococcus* strains exhibited  $\alpha$ -hemolytic activity on sheep blood agar. On the

other hand, *E. durans* MGE13.4 and *E. casseliflavus* MGM111.1 strains showed  $\beta$ - and  $\gamma$ -hemolytic activities, respectively. Among the 32 HLAR *Enterococcus* strains, only *E. faecalis* MSE61.1 and *E. avium* MSE63.1 showed gelatinase activity on Todd-Hewitt agar.

### Detection of virulence factor genes

The presence of virulence factor genes in HLAR enterococci strains were investigated by PCR using specific primers. The results of the PCR detection

**Table 1.** Primers sequences and PCR protocols used for detection of AME\* and virulence factor\*\* genes

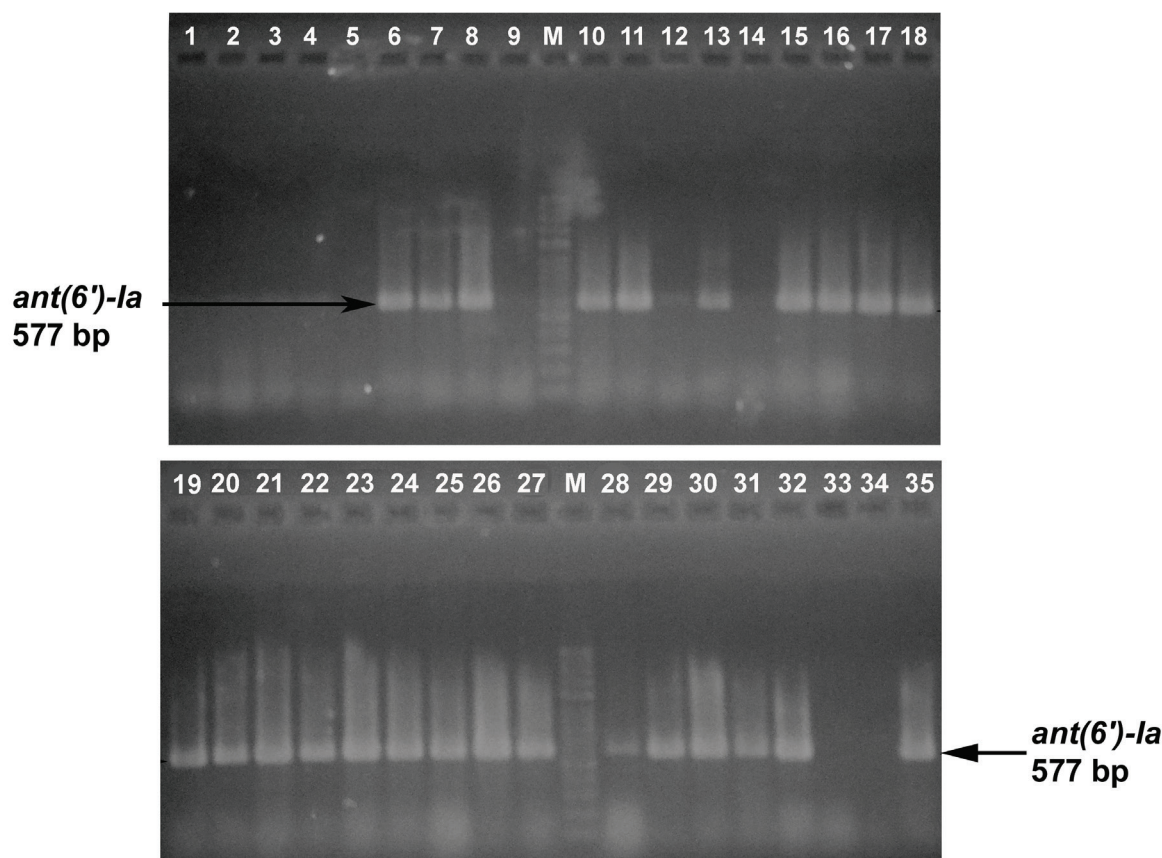
Genes	Primers sequence (5' to 3')	Product size (bp)	PCR protocol	References
<i>aph(3')-IIIa</i> *	f: GGCTAAAATGAGAATATCACCGG r: CTTTAAAAAATCATAACAGCTCGCG	523	94 °C for 3 min x1; 94 °C for 40 s, 55 °C for 40 s, 72 °C for 40 s x35; 72 °C for 2 min x1	Vakulenko et al. (2003)
<i>ant(4')-Ia</i> *	f: CAAACTGCTAAATCGGTAGAAGCC r: GGAAAGTTGACCAGACATTACGAATC	294	94 °C for 3 min x1; 94 °C for 40 s, 55 °C for 40 s, 72 °C for 40 s x35; 72 °C for 2 min x1	Vakulenko et al. (2003)
<i>ant(6')-Ia</i> *	f: ACTGGCTTAATCAATTTGGG r: GCCTTTCGCCACCTCACCG	577	94 °C for 3 min x1; 94 °C for 30 s, 56 °C for 30 s, 72 °C for 60 s x35; 72 °C for 5 min x1	Niu et al. (2016)
<i>aac(6')-Ie-aph(2'')-Ia</i> *	f: CAGGAATTTATCGAAAATGGTAGAAAAAG r: CACAATCGACTAAAGAGTACCAATC	369	94 °C for 3 min x1; 94 °C for 40 s, 55 °C for 40 s, 72 °C for 40 s x35; 72 °C for 2 min x1	Vakulenko et al. (2003)
<i>aph(2'')-Ib</i> *	f: CTTGGACGCTGAGATATAGAGCAC r: GTTGTAGCAATTCAGAAACACCCTT	867	94 °C for 3 min x1; 94 °C for 40 s, 55 °C for 40 s, 72 °C for 40 s x35; 72 °C for 2 min x1	Vakulenko et al. (2003)
<i>aph(2'')-Ic</i> *	f: CCACAATGATAATGACTCAGTTCCC r: CCACAGCTTCCGATAGCAAGAG	444	94 °C for 3 min x1; 94 °C for 40 s, 55 °C for 40 s, 72 °C for 40 s x35; 72 °C for 2 min x1	Vakulenko et al. (2003)
<i>aph(2'')-Id</i> *	f: GTGGTTTTACAGGAATGCCATC r: CCCTTTCATACCAATCCATATAACC	641	94 °C for 3 min x1; 94 °C for 40 s, 55 °C for 40 s, 72 °C for 40 s x35; 72 °C for 2 min x1	Vakulenko et al. (2003)
<i>efaA<sub>fm</sub></i> **	f: AACAGATCCGCATGAATA r: CATTTCATCATCTGATAGTA	735	95 °C for 5 min x1; 95 °C for 30 s, 54 °C for 30 s, 72 °C for 60 s x35; 72 °C for 10 min x1	Reviriego et al. (2005)
<i>efaA<sub>fs</sub></i> **	f: GACAGACCCCTCAGCAATA r: AGTTCATCATGCTGTAGTA	705	95 °C for 5 min x1; 95 °C for 30 s, 54 °C for 30 s, 72 °C for 60 s x35; 72 °C for 10 min x1	Reviriego et al. (2005)
<i>cad</i> **	f: TGCTTTGTCATTGACAATCCG r: ACTTTTCCCAACCCTCAA	1299	95 °C for 5 min x1; 95 °C for 30 s, 54 °C for 30 s, 72 °C for 60 s x35; 72 °C for 10 min x1	Reviriego et al. (2005)
<i>ccf</i> **	f: GGGAATTGAGTAGTGAAGAAG r: AGCCGCTAAAATCGGTAAAAT	543	95 °C for 5 min x1; 95 °C for 30 s, 54 °C for 30 s, 72 °C for 60 s x35; 72 °C for 10 min x1	Reviriego et al. (2005)
<i>cpd</i> **	f: TGGTGGGTATTTTTCAATTC r: TACGGCTCTGGCTACTA	782	95 °C for 5 min x1; 95 °C for 30 s, 54 °C for 30 s, 72 °C for 60 s x35; 72 °C for 10 min x1	Reviriego et al. (2005)
<i>cob</i> **	f: AACATTCAGCAAACAAAGC r: GCGTCATAAAGAGTGGTCAT	1405	95 °C for 5 min x1; 95 °C for 30 s, 54 °C for 30 s, 72 °C for 60 s x35; 72 °C for 10 min x1	Reviriego et al. (2005)
<i>esp<sub>fm</sub></i> **	f: TTGCTAATGCAAGTACGTCC r: GCATCAACACTTGCATTACCGAA	955	95 °C for 5 min x1; 95 °C for 30 s, 54 °C for 30 s, 72 °C for 60 s x35; 72 °C for 10 min x1	Reviriego et al. (2005)
<i>esp<sub>fs</sub></i> **	f: TTGCTAATGCTAGTCCACGACC r: GCGTCAACACTTGCATTGCCGAA	933	95 °C for 5 min x1; 95 °C for 30 s, 54 °C for 30 s, 72 °C for 60 s x35; 72 °C for 10 min x1	Reviriego et al. (2005)
<i>ace</i> **	f: AAAGTAGAATTAGATCCACAC r: TCTATCACATTCCGGTTGCG	350	95 °C for 5 min x1; 95 °C for 30 s, 54 °C for 30 s, 72 °C for 60 s x35; 72 °C for 10 min x1	Ben Belgacem et al. (2010)
<i>acm</i> **	f: GGCCAGAAACGTAACCGATA r: CGCTGGGAAATCTTGTAATA	353	95 °C for 5 min x1; 95 °C for 30 s, 52 °C for 30 s, 72 °C for 60 s x35; 72 °C for 10 min x1	Camargo et al. (2006)
<i>gelE</i> **	f: ACCCGGTATCATTTGGTTT r: ACGCATTGCTTTTCCATC	419	95 °C for 5 min x1; 95 °C for 30 s, 54 °C for 30 s, 72 °C for 60 s x35; 72 °C for 10 min x1	Reviriego et al. (2005)
<i>agg</i> **	f: AAGAAAAAGAAGTAGACCAAC r: AAACGGCAAGACAAGTAAATA	1553	95 °C for 5 min x1; 95 °C for 30 s, 56 °C for 30 s, 72 °C for 60 s x35; 72 °C for 10 min x1	Eaton and Gasson, (2001)
<i>cylM</i> **	f: CTGATGGAAAGAAAGATAGTAT r: TGAGTTGGTCTGATTACATTT	742	95 °C for 5 min x1; 95 °C for 30 s, 54 °C for 30 s, 72 °C for 60 s x35; 72 °C for 10 min x1	Reviriego et al. (2005)
<i>cylB</i> **	f: ATTCTACCTATGTTCTGTTA r: AATAAACTCTTCTTTCCAAC	843	95 °C for 5 min x1; 95 °C for 30 s, 54 °C for 30 s, 72 °C for 60 s x35; 72 °C for 10 min x1	Reviriego et al. (2005)
<i>cylA</i> **	f: TGGATGATAGTGATAGGAAGT r: TCTACAGTAAATCTTTCGTCA	517	95 °C for 5 min x1; 95 °C for 30 s, 54 °C for 30 s, 72 °C for 60 s x35; 72 °C for 10 min x1	Reviriego et al. (2005)
<i>hyl</i> **	f: ACAGAAGAGCTGCAGGAAATG r: GACTGACGTCCAAGTTTCCAA	276	95 °C for 2 min x1; 95 °C for 30 s, 56 °C for 90 s, 72 °C for 90 s x35; 72 °C for 10 min x1	Vankerckhoven et al. (2004)

**Table 2.** Isolation material, antibiotic resistance patterns, AME-encoding genes and virulence factor genes in HLAR *Enterococcus* strains

Strain	Isolation material	Antibiotic resistance <sup>a</sup>	MIC <sup>a,b</sup>		AME genes	Virulence factor genes
			CN	S		
<i>E. durans</i> MGE13.1	Chicken breast	DO, E, CN, QD, MH, F, S, TE	>4096 <sup>R</sup>	4096 <sup>R</sup>	<i>aac(6')-Ie-aph(2'')-Ia</i>	-
<i>E. durans</i> MGE13.2	Chicken breast	E, CN, QD, MH, F, S, TE	4096 <sup>R</sup>	4096 <sup>R</sup>	<i>ant(4')-Ia, aac(6')-Ie-aph(2'')-Ia</i>	<i>gelE, efaA<sub>fm</sub>, efaA<sub>fs</sub>, ccf, cad, cylA</i>
<i>E. durans</i> MGE13.3	Chicken breast	E, CN, QD, MH, F, S, TE	>4096 <sup>R</sup>	2048 <sup>R</sup>	<i>aac(6')-Ie-aph(2'')-Ia</i>	<i>efaA<sub>fm</sub>, cad</i>
<i>E. durans</i> MGE13.4	Chicken breast	E, CN, QD, MH, F, S, TE	>4096 <sup>R</sup>	2048 <sup>R</sup>	<i>aac(6')-Ie-aph(2'')-Ia</i>	<i>efaA<sub>fm</sub>, acm, cad</i>
<i>E. faecium</i> MSM14.1	Chicken breast	DO, E, CN, LEV, MH, RD, S, TE	4096 <sup>R</sup>	>4096 <sup>R</sup>	<i>aph(3')-IIIa, ant(4')-Ia, aac(6')-Ie-aph(2'')-Ia, aph(2'')-Ib</i>	<i>efaA<sub>fm</sub></i>
<i>E. faecium</i> MSE31.1	Chicken wing	DO, E, CN, MH, F, NOR, P, CIP, S, TE	1024 <sup>R</sup>	>4096 <sup>R</sup>	<i>aph(3')-IIIa, ant(6')-Ia, aph(2'')-Ib</i>	<i>efaA<sub>fm</sub>, acm</i>
<i>E. faecium</i> MSE53.1	Chicken breast	DO, E, CN, C, MH, S, TE	4096 <sup>R</sup>	>4096 <sup>R</sup>	<i>aph(3')-IIIa, ant(4')-Ia, ant(6')-Ia, aph(2'')-Ib, aph(2'')-Ic</i>	<i>efaA<sub>fm</sub>, acm, agg</i>
<i>E. faecalis</i> MSM53.1	Chicken breast	DO, E, CN, LEV, QD, MH, NOR, CIP, S, TE	4096 <sup>R</sup>	>4096 <sup>R</sup>	<i>aph(3')-IIIa, ant(6')-Ia, aph(2'')-Ib</i>	<i>efaA<sub>fs</sub>, cpd, cob, ccf, cad</i>
<i>E. faecium</i> MSE58.1	Chicken breast	DO, E, CN, C, LEV, MH, NOR, CIP, S, TE	>4096 <sup>R</sup>	>4096 <sup>R</sup>	<i>aph(3')-IIIa, aac(6')-Ie-aph(2'')-Ia, aph(2'')-Ib</i>	<i>efaA<sub>fm</sub>, ace, acm</i>
<i>E. faecalis</i> MGE58.1	Chicken breast	DO, E, CN, C, MH, NOR, P, CIP, S, TE	>4096 <sup>R</sup>	>4096 <sup>R</sup>	<i>aph(3')-IIIa, ant(4')-Ia, ant(6')-Ia, aph(2'')-Ib</i>	<i>gelE, efaA<sub>fm</sub>, acm</i>
<i>E. faecalis</i> MSE61.1	Chicken leg	DO, E, CN, QD, MH, S, TE	>4096 <sup>R</sup>	>4096 <sup>R</sup>	<i>aph(3')-IIIa, ant(4')-Ia, ant(6')-Ia, aph(2'')-Ib</i>	<i>gelE, efaA<sub>fs</sub>, cpd, cob, ccf, cad</i>
<i>E. durans</i> MGE63.1	Chicken wing	E, CN, C, QD, S	>4096 <sup>R</sup>	>4096 <sup>R</sup>	<i>ant(4')-Ia</i>	-
<i>E. avium</i> ME63.1	Chicken wing	E, CN, C, LEV, QD, MH, F, NOR, CIP, S, TE	4096 <sup>R</sup>	>4096 <sup>R</sup>	<i>aph(3')-IIIa, ant(6')-Ia, aac(6')-Ie-aph(2'')-Ia, aph(2'')-Ic</i>	<i>gelE, efaA<sub>fm</sub>, efaA<sub>fs</sub>, ccf, cad, cylA</i>
<i>E. avium</i> MSE63.2	Chicken wing	CN, MH, S, TEC	2048 <sup>R</sup>	>4096 <sup>R</sup>	<i>aph(3')-IIIa, ant(4')-Ia, aph(2'')-Ib, aph(2'')-Ic</i>	<i>gelE, efaA<sub>fm</sub>, acm</i>
<i>E. faecium</i> MSM76.1	Chicken wing	DO, E, LEV, MH, NOR, CIP, S, TE	32	>4096 <sup>R</sup>	<i>aph(3')-IIIa, ant(4')-Ia, ant(6')-Ia</i>	<i>gelE, efaA<sub>fm</sub>, acm</i>
<i>E. faecalis</i> MSM 93.1	Chicken wing	AMP, DO, E, C, LEV, LZD, QD, MH, NOR, P, RD, CIP, S, TEC, TE, VA	128	>4096 <sup>R</sup>	<i>aph(3')-IIIa, ant(6')-Ia,</i>	<i>gelE, efaA<sub>fm</sub>, acm</i>
<i>E. faecium</i> MSM95.1	Chicken wing	DO, E, LEV, MH, CIP, S, TE	32	>4096 <sup>R</sup>	<i>aph(3')-IIIa, ant(4')-Ia, ant(6')-Ia, aph(2'')-Ib</i>	<i>gelE, efaA<sub>fm</sub>, acm</i>
<i>E. faecium</i> MSM103.1	Chicken leg	AMP, DO, E, MH, RD, S, TE	64	>4096 <sup>R</sup>	<i>aph(3')-IIIa, ant(4')-Ia, ant(6')-Ia</i>	<i>gelE, efaA<sub>fm</sub>, acm</i>
<i>E. faecium</i> MSM104.1	Chicken leg	DO, E, MH, S, TE	64	>4096 <sup>R</sup>	<i>aph(3')-IIIa, ant(4')-Ia, ant(6')-Ia, aph(2'')-Ib</i>	<i>efaA<sub>fm</sub>, acm</i>
<i>E. faecium</i> MSE104.1	Chicken leg	DO, E, CN, LEV, MH, S, TE	>4096 <sup>R</sup>	>4096 <sup>R</sup>	<i>aph(3')-IIIa, ant(6')-Ia, aph(2'')-Ib</i>	<i>gelE, efaA<sub>fm</sub>, acm</i>
<i>E. faecium</i> MSE104.2	Chicken leg	DO, E, CN, LEV, MH, NOR, CIP, S, TE	>4096 <sup>R</sup>	>4096 <sup>R</sup>	<i>aph(3')-IIIa, ant(4')-Ia, ant(6')-Ia</i>	<i>efaA<sub>fm</sub>, acm</i>
<i>E. faecium</i> MSM105.1	Chicken leg	DO, E, QD, MH, CIP, S, TE	64	>4096 <sup>R</sup>	<i>aph(3')-IIIa, ant(4')-Ia, ant(6')-Ia, aph(2'')-Ib</i>	<i>gelE, efaA<sub>fm</sub>, acm</i>
<i>E. faecium</i> MSM106.1	Chicken wing	DO, E, MH, RD, S, TE	64	>4096 <sup>R</sup>	<i>aph(3')-IIIa, ant(6')-Ia, aph(2'')-Ib</i>	<i>efaA<sub>fm</sub>, acm</i>
<i>E. faecium</i> MSM107.1	Chicken leg	DO, E, MH, CIP, S, TE	32	>4096 <sup>R</sup>	<i>aph(3')-IIIa, ant(4')-Ia, ant(6')-Ia, aph(2'')-Ib</i>	<i>gelE, efaA<sub>fm</sub>, acm, cylA</i>
<i>E. faecium</i> MSM108.1	Chicken leg	DO, E, MH, S, TE	32	>4096 <sup>R</sup>	<i>aph(3')-IIIa, ant(4')-Ia, ant(6')-Ia, aph(2'')-Ib</i>	<i>efaA<sub>fm</sub>, acm</i>
<i>E. faecium</i> MSM109.1	Chicken breast	DO, E, MH, RD, S, TE	32	>4096 <sup>R</sup>	<i>aph(3')-IIIa, ant(4')-Ia, ant(6')-Ia</i>	<i>efaA<sub>fm</sub>, cpd, ccf, acm, cylA</i>
<i>E. faecium</i> MSM110.1	Chicken wing	DO, E, MH, NOR, RD, CIP, S, TE	32	>4096 <sup>R</sup>	<i>aph(3')-IIIa, ant(4')-Ia, ant(6')-Ia, aph(2'')-Ib</i>	<i>efaA<sub>fm</sub>, acm</i>
<i>E. casseliflavus</i> MGM111.1	Chicken wing	E, CN, C, MH, TE	4096 <sup>R</sup>	64	<i>ant(4')-Ia, ant(6')-Ia, aph(2'')-Ib</i>	<i>efaA<sub>fm</sub></i>
<i>E. avium</i> MSE111.1	Chicken wing	E, CN, MH, S, TE	512 <sup>R</sup>	4096 <sup>R</sup>	<i>aph(3')-IIIa, ant(6')-Ia, aph(2'')-Ib</i>	<i>efaA<sub>fm</sub></i>
<i>E. faecium</i> MSE111.2	Chicken wing	E, LEV, QD, MH, S, TE	256 <sup>R</sup>	>4096 <sup>R</sup>	<i>aph(3')-IIIa, ant(4')-Ia, ant(6')-Ia</i>	<i>efaA<sub>fm</sub>, acm</i>
<i>E. faecalis</i> MSM112.1	Chicken drumstick	DO, E, QD, MH, NOR, CIP, S, TE	16	2048 <sup>R</sup>	<i>aph(3')-IIIa, ant(4')-Ia, ant(6')-Ia, aph(2'')-Ib</i>	<i>efaA<sub>fm</sub>, acm</i>
<i>E. faecium</i> MSM112.2	Chicken drumstick	DO, E, LEV, MH, RD, CIP, S, TE	64	>4096 <sup>R</sup>	<i>aph(3')-IIIa, ant(4')-Ia, ant(6')-Ia, aph(2'')-Ib</i>	<i>efaA<sub>fm</sub></i>

<sup>a</sup>These data were taken from a previous study by Yalçın & Tuncer (2021). AMP, Ampicillin; C, Chloramphenicol; CIP, Ciprofloxacin; DO, Doxycycline; E, Erythromycin; CN, Gentamicin; LEV, Levofloxacin; LZD, Linezolid; MH, Minocycline; NOR, Norfloxacin; P, Penicillin G; QD, Quinupristin/dalfopristin; RD, Rifampin; S, Streptomycin; TE, Tetracycline; TEC, Teicoplanin; VA, Vancomycin.

<sup>b</sup>Antibiotics were diluted in a concentration range of 0.125 to 4096 µg/mL. Susceptibility or resistance of HLAR *Enterococcus* were determined according to the guidelines of CLSI (2016) and EUCAST (2018).



**Figure 1.** PCR amplification of *ant(6')-Ia* in HLAR *Enterococcus* strains. Line 1: *E. durans* MGE13.1; line 2: *E. durans* MGE13.2; line 3: *E. durans* MGE13.3; line 4: *E. durans* MGE13.4; line 5: *E. faecium* MSM14.1; line 6: *E. faecium* MSE31.1; line 7: *E. faecium* MSE53.1; line 8: *E. faecalis* MSM53.1; line 9: *E. faecium* MSE58.1; line 10: *E. faecalis* MGE58.1; line 11: *E. faecalis* MSE61.1; line 12: *E. durans* MGE63.1; line 13: *E. avium* MSE63.1; line 14: *E. avium* MSE63.2; line 15: *E. faecium* MSM76.1; line 16: *E. faecalis* MSM 93.1; line 17: *E. faecium* MSM95.1; line 18: *E. faecium* MSM103.1; line 19: *E. faecium* MSM104.1; line 20: *E. faecium* MSE104.1; line 21: *E. faecium* MSE104.2; line 22: *E. faecium* MSM105.1; line 23: *E. faecium* MSM106.1; line 24: *E. faecium* MSM107.1; line 25: *E. faecium* MSM108.1; line 26: *E. faecium* MSM109.1; line 27: *E. faecium* MSM110.1; line 28: *E. casseliflavus* MGM111.1; line 29: *E. avium* MSE111.1; line 30: *E. faecium* MSE111.2; line 31: *E. faecalis* MSM112.1; line 32: *E. faecium* MSM112.2; line 33: *E. faecalis* ATCC 29212 (negative control); line 34: negative control (water) (negative control); line 35: *E. faecium* ATCC 51559 (positive control); line M: 100 bp DNA ladder (Thermo Scientific)

**Table 3.** AME-encoding gene pattern types and distributions of AME-encoding gene patterns in HLAR *Enterococcus* strains

AME-encoding gene pattern type	AME-encoding genes	<i>E. faecium</i>	<i>E. faecalis</i>	<i>E. durans</i>	<i>E. avium</i>	<i>E. casseliflavus</i>	Total
		(n= 18) n (%)	(n= 5) n (%)	(n= 5) n (%)	(n= 3) n (%)	(n= 1) n (%)	(n= 32) n (%)
I	<i>aph(3')-IIIa+ant(4')-Ia+ant(6')-Ia+aph(2'')-Ib</i>	8 (44.44%)	3 (60%)				11 (34.38%)
II	<i>aph(3')-IIIa+ant(4')-Ia+ant(6')-Ia</i>	5 (27.78%)					5 (15.63%)
III	<i>aph(3')-IIIa+ant(6')-Ia+aph(2'')-Ib</i>	3 (16.67%)	1 (20%)		1 (33.33%)		5 (15.63%)
IV	<i>ant(6')-Ia+aac(6')-Ie-aph(2'')-Ia</i>			3 (60%)			3 (9.38%)
V	<i>ant(4')-Ia</i>			1 (20%)			1 (3.13%)
VI	<i>aph(3')-IIIa+ant(6')-Ia</i>		1 (20%)				1 (3.13%)
VII	<i>ant(4')-Ia+ant(6')-Ia+aph(2'')-Ib</i>					1 (100%)	1 (3.13%)
VIII	<i>ant(4')-Ia+ant(6')-Ia+aac(6')-Ie-aph(2'')-Ia</i>			1 (20%)			1 (3.13%)
IX	<i>aph(3')-IIIa+aac(6')-Ie-aph(2'')-Ia+aph(2'')-Ib</i>	1 (5.56%)					1 (3.13%)
X	<i>aph(3')-IIIa+ant(4')-Ia+aac(6')-Ie-aph(2'')-Ia+aph(2'')-Ib</i>	1 (5.56%)					1 (3.13%)
XI	<i>aph(3')-IIIa+ant(4')-Ia+aph(2'')-Ib+aph(2'')-Ic</i>				1 (33.33%)		1 (3.13%)
XII	<i>ant(4')-Ia+ant(6')-Ia+aac(6')-Ie-aph(2'')-Ia+aph(2'')-Ic</i>				1 (33.33%)		1 (3.13%)



of virulence factor genes in HLAR enterococci are shown in Table 2. HLAR strains, except *E. durans* MGE13.1 and MGE63.1, contain at least one virulence factor gene. The *efaA<sub>fm</sub>* (87.5%, 28/32), *acm* (65.63%, 21/32) and *gelE* (37.5%, 12/32) were found to be the most prevalent virulence factor genes in HLAR strains. In addition, the *cad*, *ccf*, *cylA*, *efaA<sub>fs</sub>*, *cpd*, *cob*, *agg* and *ace* were found in 18.75% (6/32), 16.63% (5/32), 12.5% (4/32), 12.5% (4/32), 9.38% (3/32), 6.25% (2/32), 3.13% (1/32) and 3.13% (1/32) of strains, respectively. The other virulence factor genes, *esp<sub>fm</sub>*, *esp<sub>fs</sub>*, *cylM*, *cylB* and *hyl*, were not detected in any of the strains used in this study.

## DISCUSSION

Enterococci are frequent contaminants of poultry meat due to the lower hygienic standards in poultry slaughtering (Bortolaia et al., 2016). Previous studies reported that different *Enterococcus* species are isolated from retail chicken meat samples in USA (Donabedian et al., 2003), South Korea (Kim et al., 2018; Kim et al., 2019) and Türkiye (Yılmaz et al., 2016; Onaran et al., 2019; Yalçın and Tuncer, 2021). It was showed that some of these strains are HLAR (Donabedian et al., 2003; Kim et al., 2019; Yalçın and Tuncer, 2021). The presence of AME-encoding genes is the primary cause of high-level aminoglycoside resistance (Niu et al., 2016). The high-level streptomycin resistance in enterococci is generally associated with *ant(6')-Ia* and *aph(3')-IIIa* genes (Niu et al., 2016; Özdemir and Tuncer, 2020). In our study, 27 of 31 HLSR *Enterococcus* strains carried at least one of these two AME-encoding genes. Similar to our results, the *ant(6')-Ia* and/or *aph(3')-IIIa* are the most commonly detected in HLSR enterococci isolated from clinical samples (Niu et al., 2016), ready-to-eat meat samples (Chajęcka-Wierzchowska et al., 2016), retail chicken meat (Kim et al., 2019), and raw milk and traditional Turkish cheeses (Özdemir and Tuncer, 2020). On the other hand, the presence of *ant(6')-Ia* and/or *aph(3')-IIIa* genes was not detected in four *E. durans* MGE13.1, MGE13.2, MGE13.3 and MGE13.4 strains that were phenotypically high-level streptomycin resistant. The high-level streptomycin resistance in these strains is thought to result from a different AME-encoding mechanism (Ramirez and Tolmasky, 2010; Peyvasti et al., 2020). Moreover, the *ant(6')-Ia* gene was found in HLGR *E. casseliflavus* MGM111.1 strain although it was phenotypically susceptible to streptomycin. Similar to our result, Choi and Woo (2013) reported that one of their HLGR

enterococci isolates was susceptible to streptomycin even though it was carrying *ant(6')-Ia*.

The *aac(6')-Ie-aph(2'')-Ia*, *aph(2'')-Ib*, *aph(2'')-Ic* and *aph(2'')-Id* genes are responsible for high-level gentamicin resistance in enterococci (Niu et al., 2016; Shete et al., 2017). In our study, it was determined that 16 out of 19 HLGR *Enterococcus* strains contained at least one of the *aac(6')-Ie-aph(2'')-Ia*, *aph(2'')-Ib* and *aph(2'')-Ic* genes. The most common detected AME-encoding gene among these three genes in HLGR *Enterococcus* strains was found as the *aph(2'')-Ib* (11/19, 57.90%). The *aph(2'')-Ib* gene was followed by *aac(6')-Ie-aph(2'')-Ia* (7/19, 36.84%) and *aph(2'')-Ic* (3/19, 15.79%) genes. Contrary to our results, recent research has shown that high-level gentamicin resistance in enterococci is predominantly associated with the presence of bifunctional *aac(6')-Ie-aph(2'')-Ia* gene (Choi and Woo, 2013; Li et al., 2015; Jaimee and Halami, 2016; Niu et al., 2016; Shete et al., 2017; Amini et al., 2018; Ramin et al., 2018; Kim et al., 2019; Peyvasti et al., 2020). On the other hand, Özdemir and Tuncer (2020) indicated that none of the 54 HLGR enterococci strains did contain *aac(6')-Ie-aph(2'')-Ia* or *aph(2'')-Ib* genes but 10 of 54 HLGR strains contained *aph(2'')-Ic* gene. The *aph(2'')-Id* gene was detected none of the 32 HLAR *Enterococcus* strains, as previously reported by Choi and Woo (2013), Padmasini et al. (2014), Shete et al. (2017), Amini et al. (2018) and Özdemir and Tuncer (2020). However, the presence of a low rate of *aph(2'')-Id* gene in HLAR enterococci isolated from various sources was reported by different researchers (Donabedian et al., 2003; Harada et al., 2004; Jackson et al., 2004; Li et al., 2015; Chajęcka-Wierzchowska et al., 2016; Nowakiewicz et al., 2017).

Gelatinase is an extracellular metalloendopeptidase encoded by the *gelE* gene located on chromosome. Potentially contributing to virulence, this enzyme hydrolyzes gelatin, elastin, collagen, hemoglobin and other bioactive peptides (Chajęcka-Wierzchowska et al., 2017). Previous researches showed that gelatinase activity is more commonly detected in both clinical and food isolates of *E. faecalis* than other enterococci species (Eaton and Gasson, 2001; Semedo et al., 2003). In this study, gelatinase activity was detected in only two HLAR *Enterococcus* strains. To our knowledge, there are limited study investigating gelatinase activity in HLAR enterococci strains (Han et al., 2011; Adifon and Tuncer, 2019). Contrary to our results, Han et al. (2011) high frequently detect-



ed gelatinase activity in HLAR enterococci isolated from broiler feces in South Korea. However, Adifon and Tuncer (2019) reported that only three *E. faecalis* strains among 54 HLAR enterococci isolated from traditional Turkish Tulum cheeses showed gelatinase activity, as confirmed in this study.

Hemolysin/cytolysin, a bacterial toxin, is one of the virulence factors secreted by enterococci. Hemolytic activity, which can be encoded by plasmid or chromosomal DNA, plays an important role in increasing the severity of infection.  $\beta$ -hemolytic activity is mostly observed in clinical isolates of *E. faecium* and *E. faecalis* species. It is not desirable to isolate enterococci with  $\beta$ -hemolytic activity from foods (Semedo et al., 2003). In this study, only the *E. durans* MGE13.4 strain was found to have  $\beta$ -hemolytic activity, while the majority of the HLAR *Enterococcus* strains had  $\alpha$ -hemolytic activity (93.75%). Similar to our results, Adifon and Tuncer (2019) reported that only three of 54 (5.55%) HLAR enterococci strains from traditional Turkish cheeses were shown  $\beta$ -hemolytic activity. Researchers also stated that 46.30% (25/54) and 48.15% (26/54) of their HLAR isolates were  $\gamma$ - and  $\alpha$ -hemolytic, respectively.

The most common virulence factor genes in HLAR *Enterococcus* strains were found to be *efaA<sub>fm</sub>* (87.5%, 28/32), *acm* (65.63%, 21/32) and *gelE* (37.5%, 12/32). Similar to our results, Kim et al. (2019) reported that HLAR *E. faecium* and *E. faecalis* strains isolated from retail chicken meat in South Korea contain mostly *efaA* and *gelE* genes. Researchers also stated that *ace* (a collagen-binding protein) and *asal* (aggregation substance) were the other most common detected virulence genes in their HLAR isolates, as conversely to our results. In our study, although the *ace* gene was detected very low rate in HLAR enterococci strains, distinct collagen-binding protein-encoding gene *acm* was detected in 21 of 32 strains. Also, different researchers stated that *efaA* (26.7% and 85.19%) and *gelE* (33.3% and 59.2%) genes were commonly found

in HLAR enterococci isolated from clinical samples (Niu et al., 2016) and traditional cheeses (Adifon and Tuncer, 2019), respectively. In addition, Adifon and Tuncer (2019) indicated that *ccf* (88.89%), *acm* (77.78%), *cpd* (51.85%) and *esp<sub>fs</sub>* (50%) were other most prevalent virulence factor genes in their HLAR enterococci isolates. In another similar study, Kang et al. (2021) reported that HLAR *E. faecalis* strains isolated from bulk tank milk in Korea exhibited a high prevalence of virulence genes such as *ace* (99.5%), *efaA* (98.9%), *cad1* (98.4%), *gelE* (85.9%), and *asal* (61.6%).

## CONCLUSION

This study indicates that HLAR *Enterococcus* strains isolated from retail chicken meat in Türkiye contain various AME-encoding genes and virulence factor genes. The *aph(2'')-Ib* and *aph(3')-IIIa/ant(6')-Ia* genes were found to be the most common AME-encoding genes in HLGR and HLSR enterococci strains, respectively. At least one virulence factor gene was detected in HLAR enterococci strains, except *E. durans* MGE13.1 and MGE63.1. The most prevalent virulence factor genes in HLAR strains were detected as *efaA<sub>fm</sub>*, *acm* and *gelE*. In conclusion, HLAR enterococci strains containing the AME-encoding genes may serve as reservoirs for the spread of high-level aminoglycoside resistance among bacteria. In addition, HLAR enterococci strains that have the virulence factor genes may pose a risk to consumer health.

## ACKNOWLEDGMENTS

This study was supported by project no. 5010-YL1-17 from the Scientific Research Projects Coordination Unit of Süleyman Demirel University (Isparta, Türkiye).

## CONFLICT OF INTEREST

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

## REFERENCES

- Abuelnaga M, Lamas A, Quintela-Baluja M, Osman M, Miranda JM, Cepeda A, Franco CM (2016) Evaluation of the extent of virulence factors and antibiotic resistance in enterococci isolated from fermented and unfermented foods. *Ann Microbiol* 66:577-585.
- Adifon E, Tuncer Y (2019) Investigation of virulence factors using phenotypic and genotypic methods in high-level aminoglycoside resistant enterococci isolated from cheese. *Gida*, 44(4):719-732.
- Akpinar Kankaya D and Tuncer Y (2022) Detection of virulence factors, biofilm formation and biogenic amine production in vancomycin-resistant lactic acid bacteria (VRLAB) isolated from foods of animal origin. *J Food Process Preserv* 46:e16423.
- Amini F, Krimpour HA, Ghaderi M, Vaziri S, Ferdowsi S, Azizi M, Amini S (2018) Prevalence of aminoglycoside resistance genes in *Enterococcus* strains in Kermanshah, Iran. *Iran J Med Sci* 43(5):487-493.
- Arias CA and Murray BE (2012) The rise of the *Enterococcus*: beyond vancomycin resistance. *Nat Rev Microbiol* 10:266-278.
- Ben Belgacem Z, Abriouel H, Omar NB, Lucas R, Martinez-Canamero M, Galvez A, Manai M (2010) Antimicrobial activity, safety aspects, and some technological properties of bacteriocinogenic *Enterococcus faecium* from artisanal Tunisian fermented meat. *Food Control* 21(4):462-470.
- Bortolaia V, Espinosa-Gongora C, Guardabassi L (2016) Human health risks associated with antimicrobial-resistant enterococci and *Staphylococcus aureus* on poultry meat. *Clin Microbiol Infect* 22(2):130-140.
- Camargo ILBC, Gilmore MS, Darini ALC (2006) Multilocus sequence typing and analysis of putative virulence factors in vancomycin-resistant and vancomycin-sensitive *Enterococcus faecium* isolates from Brazil. *Clin Microbiol Infect* 12(11):1123-1130.
- Cancilla MR, Powell LB, Hillier AJ, Davidson BE (1992) Rapid genomic fingerprinting of *Lactococcus lactis* strains by arbitrarily primed polymerase chain reaction with 32P and fluorescent labels. *Appl Environ Microbiol* 58(5):1772-1775.
- Cariolato D, Andrighetto C, Lombardi A (2008) Occurrence of virulence factors and antibiotic resistances in *Enterococcus faecalis* and *Enterococcus faecium* collected from dairy and human samples in North Italy. *Food Control* 19(9):886-892.
- Chajęcka-Wierzchowska W, Zadernowska A, Łaniewska-Trokenheim Ł (2016) Diversity of antibiotic resistance genes in *Enterococcus* strains isolated from ready-to-eat meat products. *J Food Sci* 81(11):M2799-M2807.
- Chajęcka-Wierzchowska W, Zadernowska A, Łaniewska-Trokenheim Ł (2017) Virulence factors of *Enterococcus* spp. presented in food. *LWT-Food Sci Technol* 75:670-676.
- Chajęcka-Wierzchowska W, Zadernowska A, Mónica García-Solache M (2020) Ready-to-eat dairy products as a source of multidrug-resistant *Enterococcus* strains: phenotypic and genotypic characteristics. *J Dairy Sci* 103(5):4068-4077.
- Choi J-M and Woo G-J (2013) Molecular characterization of high-level gentamicin-resistant *Enterococcus faecalis* from chicken meat in Korea. *Int J Food Microbiol* 165(1):1-6.
- Donabedian SM, Thal LA, Hershberger E, Perri MB, Chow JW, Bartlett P, Jones R, Joyce K, Rossiter S, Gay K, Johnson J, Mackinson C, Debess E, Madden J, Angulo F, Zervos MJ (2003) Molecular characterization of gentamicin-resistant *Enterococci* in the United States: evidence of spread from animals to humans through food. *J Clin Microbiol* 41(3):1109-1113.
- Eaton TJ and Gasson MJ (2001) Molecular screening of *Enterococcus* virulence determinants and potential for genetic exchange between food and medical isolates. *Appl Environ Microbiol* 67(4):1628-1635.
- Guzman Prieto AM, van Schaik W, Rogers MRC, Coque TM, Baquero F, Corander J, Willems RJL (2016) Global emergence and dissemination of enterococci as nosocomial pathogens: attack of the clones? *Front Microbiol* 7:788.
- Han D, Unno T, Jang J, Lim K, Lee SN, Ko G, Sadowsky MJ, Hur HG (2011) The occurrence of virulence traits among high-level aminoglycosides resistant *Enterococcus* isolates obtained from feces of humans, animals, and birds in South Korea. *Int J Food Microbiol* 144(3):387-392.
- Harada T, Mito Y, Otsuki K, Murase T (2004) Resistance to gentamicin and vancomycin in enterococcal strains isolated from retail broiler chickens in Japan. *J Food Prot* 67(10):2292-2295.
- Hollenbeck BL and Rice LB (2012) Intrinsic and acquired resistance mechanisms in enterococcus. *Virulence* 3(5):421-433.
- Hugas M, Garriga M, Aymerich MT (2003) Functionality of enterococci in meat products. *Int J Food Microbiol* 88:223-233.
- Inoğlu Z and Tuncer Y (2013) Safety assessment of *Enterococcus faecium* and *Enterococcus faecalis* strains isolated from Turkish tulum cheese. *J Food Saf* 33:369-377.
- Jackson CR, Fedorka-Cray PJ, Barrett JB, Lately SR (2004) Genetic relatedness of high-level aminoglycoside-resistant enterococci isolated from poultry carcasses. *Avian Dis* 48(1):100-107.
- Jaimee G and Halami PM (2016) High level aminoglycoside resistance in *Enterococcus*, *Pediococcus* and *Lactobacillus* species from farm animals and commercial meat products. *Ann Microbiol* 66:101-110.
- Kang HJ, Yoon S, Kim K, Lee YJ (2021) Characteristics of high-level aminoglycoside resistant *Enterococcus faecalis* isolated from bulk tank milk in Korea. *Animals* 11(6):1724.
- Kim YB, Seo KH, Son SH, Noh EB, Lee YJ (2019) Genetic characterization of high-level aminoglycoside-resistant *Enterococcus faecalis* and *Enterococcus faecium* isolated from retail chicken meat. *Poult Sci* 98:5981-5988.
- Kim Y-J, Park J-H, Seo KH (2018) Comparison of the loads and antibiotic-resistance profiles of *Enterococcus* species from conventional and organic chicken carcasses in South Korea. *Poult Sci* 97:271-278.
- Li W, Li J, Wei Q, Hu Q, Lin X, Chen M, Ye R, Lv H (2015) Characterization of aminoglycoside resistance and virulence genes among *Enterococcus* spp. isolated from a hospital in China. *Int J Environ Res Public Health* 12(3):3014-3025.
- Niu H, Yu H, Hu T, Tian G, Zhang L, Guo X, Hu H, Wang Z (2016) The prevalence of aminoglycoside-modifying enzyme and virulence genes among enterococci with high-level aminoglycoside resistance in Inner Mongolia, China. *Braz J Microbiol* 47(3):691-696.
- M'hir S, Minervini F, Di Cagno R, Chammem N, Hamdi M (2012) Technological, functional and safety aspects of enterococci in fermented vegetable products: a mini-review. *Ann Microbiol* 62:469-481.
- Nowakiewicz A, Ziółkowska G, Trościańczyk A, Zieba P, Gnat S (2017) Determination of resistance and virulence genes in *Enterococcus faecalis* and *E. faecium* strains isolated from poultry and their genotypic characterization by ADSRRS-fingerprinting. *Poult Sci* 96(4):986-996.
- Onaran B, Göncüoğlu M, Bilir Ormancı FS (2019) Antibiotic resistance profiles of vancomycin resistant enterococci in chicken meat samples. *Ankara Üniv Vet Fak Derg* 66(4):331-336.
- Özdemir R and Tuncer Y (2020) Detection of antibiotic resistance profiles and aminoglycoside-modifying enzyme (AME) genes in high-level aminoglycoside-resistant (HLAR) enterococci isolated from raw milk and traditional cheeses in Turkey. *Mol Biol Rep* 47(3):1703-1712.
- Padmasini E, Padmaraj R, Ramesh SS (2014) High level aminoglycoside resistance and distribution of aminoglycoside resistant genes among clinical isolates of *Enterococcus* species in Chennai, India. *Sci World J Article ID:329157*.
- Peyvasti VS, Mobarez AM, Shahcheraghi F, Khoramabadi N, Rahmati NR, Doust RH (2020) High-level aminoglycoside resistance and distribution of aminoglycoside resistance genes among *Enterococcus* spp. clinical isolates in Tehran, Iran. *J Glob Antimicrob Resist* 20:318-323.
- Ramin B, Asadpour L, Tehrani HF, Amirmozafari N (2018) Detection and distribution of various HLAR gene in *Enterococcus faecalis* and *Enterococcus faecium* by multiplex-PCR. *Mod Med Lab J* 1(2):68-76.
- Ramirez MS and Tolmasky ME (2010) Aminoglycoside modifying enzymes. *Drug Resist Updates* 13(6):151-171.

- Reviriego C, Eaton T, Martín R, Jiménez E, Fernández L, Gasson MJ, Rodríguez JM (2005) Screening of virulence determinants in *Enterococcus faecium* strains isolated from breast milk. *J Hum Lact* 21(2):131-137.
- Semedo T, Santos MA, Lopes MF, Marques JFF, Crespo MT, Tenreiro R (2003) Virulence factors in food, clinical and reference enterococci: a common trait in the genus. *Syst Appl Microbiol* 26(1):13-22.
- Shete V, Grover N, Kumar M (2017) Analysis of aminoglycoside modifying enzyme genes responsible for high-level aminoglycoside resistance among enterococcal isolates. *J Pathog* Article ID:3256952.
- Vakulenko SB, Donabedian SM, Voskresenskiy AM, Zervos MJ, Lerner SA, Chow JW (2003) Multiplex PCR for detection of aminoglycoside resistance genes in enterococci. *Antimicrob Agents Chemother* 47(4):1423-1426.
- Vankerckhoven V, Autgaerden TV, Vael C, Lammens C, Chapelle S, Rossi R, Jabes D, Goossens H (2004) Development of a multiplex PCR for the detection of *asa1*, *gelE*, *cyfA*, *esp*, and *hyl* genes in enterococci and survey for virulence determinants among European hospital isolates of *Enterococcus faecium*. *J Clin Microbiol* 42(10):4473-4479.
- Yalçın M and Tuncer Y (2021) Determination of the antibiotic resistance profiles of high-level aminoglycoside resistant enterococci isolated from broiler meat. *Gıda*, 46(4):803-816.
- Yılmaz EŞ, Aslantaş Ö, Önen SP, Türkyılmaz S, Kürekci C (2016) Prevalence, antimicrobial resistance and virulence traits in enterococci from food of animal origin in Turkey. *LWT-Food Sci Technol* 66:20-26.
- Yogurtcu NN and Tuncer Y (2013) Antibiotic susceptibility patterns of *Enterococcus* strains isolated from Turkish Tulum cheese. *Int J Dairy Technol* 66(2):236-242.