Vancomycin and high-level aminoglycoside resistance in *Enterococcus species*

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Abstract

The aim of the study was to investigate vancomycin and high-level aminoglycoside resistance (HLAR) in *Enterococcus* species by phenotypic and genotypic methods. A hundred *Enterococcus* strains were included in the study. Antimicrobial susceptibilities of strains were investigated by automated system, beta-lactamase production was investigated by nitrocefin disks, vancomycin resistance and HLAR were investigated by gradient diffusion method (GDM) and disk diffusion method, respectively. For detection of vancomycin and high-level gentamicin resistance (HLGR) genes, polymerase chain reaction was used. Teicoplanin linezolid, vancomycin, ampicillin, penicillin are the most susceptible antibiotics and strains were detected not to produce beta lactamase. Vancomycin resistance was detected in ten isolates by automated system and in only five isolates by GDM. Five isolates carrying *VanA* gene were determined. The ratio of HLGR and high-level streptomycin resistance was found 40 and 63% respectively. *aac (6')-Ie-aph (2'')-Ia* gene was detected in 58% of strains. *E. faecium* strains were found more resistant to the antibiotics than the other species. Beta lactamase was detected in none of strains. The automated system detected vancomycin resistance in more strains than GDM. Therefore it is concluded that strains, which were detected to be resistant to vancomycin, should be confirmed by GDM. The ratio of *VanA* gene in strains is consistent with other studies. The HLAR ratio was found in about half of strains. The ratio of *aac(6')-Ie-aph(2'')-Ia* gene, which is the most reported gene in our country and other countries and one of the HLGR genes investigated in our study, was detected 58%.

Introduction

Enterococcus species are detected with increasing frequency in the etiology of nosocomial infections. *Enterococcus faecalis* and *Enterococcus faecium* are the most common causes among nosocomial urinary tract infections, surgical site infections and bacteraemia and they lead to serious infections such as endocarditis.[1]

Treatment of enterococcal infections is difficult because they are resistant to many antimicrobial agents by intrinsically and some types of these bacteria show multiple drug resistance. The intrinsic penicillin resistance in enterococci is depended on the presence of penicillin-binding protein 5 (PBP-5) enzyme, which shows low binding affinity to beta-lactam antibiotics. Therefore enterococci are resistant to many beta-lactam antibiotics. The other mechanism of resistance to beta-lactam antibiotics is the production of beta-lactamase. Beta-lactamase-producing enterococci are rarely isolated.[2] Beta lactamase-producing *E. faecium* strain was first identified in 1981 in the United States.[3]

Enterococci develop resistance to aminoglycoside by two different mechanisms. The moderate level of resistance usually develops due to low permeability. This type of resistance can be eliminated by using aminoglycoside with beta lactam group antibiotics, which inhibit cell wall synthesis. High-level resistance (HLR) occurs due to the result of changes in the ribosome binding site of aminoglycosides or the synthesis of enzymes that inactivate aminoglycoside. HLR is often dependent on the production of transferable plasmid-mediated aminoglycoside inactivating enzymes. The most common aminoglycoside-modifying enzyme in enterococci is APH (2'')-AAC (6') and this enzyme is encoded by *aac(6') - aph(2'')* genes and consists of two enzymes fused together. This enzyme is responsible for resistance to all aminoglycosides except streptomycin. Aminoglycoside-modifying enzymes are mainly responsible from high-level streptomycin resistance.[4,5] So far detected aminoglycoside resistance genes in enterococci encoding aminoglycoside-modifying enzymes are *aac (6')-Ie-aph (2'')-Ia, aph(2'')-Ib, aph(2'')-Ic, aph(2'')-Id, aph(3')-IIIa, aac(6')-Ii, ant(3'')-Ia, ant(4')-Ia, ant(6')-Ia*. The aminoglycoside resistance in enterococci leads to abortion of treatment wherefore this resistance causes elimination of synergistic effect between beta-lactams and aminoglycosides.

As well as, recently major problems have been encountered in the treatment of emerging vancomycin-resistant enterococci (VRE).[4] New pathogens resistant to vancomycin generally lead to difficulty in treatment because they are also resistant to other antibiotics. Resistance to glycopeptide group antibiotics was first reported in 1988 and then high-level vancomycin-and teicoplanin-resistant strains have spread worldwide.[6]

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In studies, glycopeptide resistant enterococci have fairly wide geographical spread and both genotypic and phenotypic heterogenity was determined. So far seven-vancomycin resistance phenotype are defined. These are VanA, VanB, VanC, VanD, VanE, VanG, VanL.[7] VanA and VanB resistance phenotypes have been described in *E. faecalis* and *E. faecium*. VanA resistant strains can be induced and show high-level resistance to vancomycin and teicoplanin.

In this study, the investigation of vancomycin and high-level aminoglycoside resistance (HLAR) in 100 *Enterococcus* strains isolated from clinical samples (urine, wound, abscess blood) in Microbiology Laboratory of Mustafa Kemal University Hospital between January 2008-August 2011, by phenotypic and genotypic methods was aimed.

Materials and Methods

A hundred *Enterococcus* strains were included in the study isolated from clinical samples (urine, wounds, abscess, blood) between January 2008 and August 2011 in Microbiology Laboratory of Mustafa Kemal University Hospital. *Staphylococcus aureus* ATCC 25923 and *Enterococcus faecalis* ATCC 29212 were

used as control strains.

Identification and antimicrobial susceptibility of the strains were determined by Vitek 2 automated system (bioMerieux, Mercy L'Etoile, France). HLAR was investigated by disk diffusion test using gentamicin (120 µg) and streptomycin (300 µg) disks (Becton Dickinson, Franklin Lakes, NJ, USA). And it was evaluated according to Clinical and Laboratory Standards Institute (CLSI) criteria.[8] Vancomycin MIC values of the strains were determined by gradient diffusion method (GDM) using E-test strips (BioMerieux, France) and evaluated according to CLSI criteria.[8] Nitrocefin method and the nitrocefin disks (Becton Dickinson, USA) were used to investigate the presence of the beta-lactamase in the strains.

Investigation of vancomycin and high-level aminoglycoside genes by polymerase chain reaction

DNA isolation

Bacterial DNA isolation was performed with commercial DNA extraction kit [High Pure polymerase chain reaction (PCR) Template Preparation Kit (Roche, Basel, Switzerland)] according to manufacturer's directions.

Amplifying of VanA, VanB and Van C genes

Amplification of *VanA, VanB, Van C* genes were performed by PCR method, using primers which were reported by Aktas and colleagues,[9] *aac(6')-1e-aph(2'')-1a, aph(2'')-1b, aph(2'')-1c, aph(2'')-1d* genes were performed using primers which were reported by Qu *et al*.[10] (Table 1). The PCR amplification was carried out in a total volume of 25 µL reaction mixture. The reaction mixture consisted of 2.5 µL Taq buffer (10×) (Fermentas, Waltham, MS, USA), 1.5 µL $MgCl_2$ (25 mm) (Fermentas, USA), 0.5 µL dNTP (10 mM) (Fermentas, USA), 0.25 µL (50 pmol) of each primer, 0.5 U Taq polymerase (Fermentas, USA) and 2.5 µL DNA and brought up to a 25 µL final volume with distilled water. All the amplification processes in this study were started with an initial denaturation step (94°C, 5 min). All the PCR reaction in this study consisted of 30 cycles of amplification. The other steps and temperatures were shown in Table 2.

Amplifying the gentamycin resistance genes

For the amplification of *aac(6')-1e-aph(2'')-1a, aph(2'')-1b* and *aph(2'')-1c, aph(2'')-1d* genes, 25 µL amplification mixture containing 16.6 µL distilled water, 2.5 µL 10X PCR buffer, 1.5 µL Mg_2Cl (25 mm), 0.5 µL dNTP (10 mm) mixture, 0.3 µL each primer (50 pmol), 0.3 U Taq DNA polymerase (Fermentas, USA), 3 µL extracted DNA. The steps of PCR and temperatures were shown in Table 2.

Demonstration of PCR products

The PCR products were analyzed in a 2% (w/v) agarose gel in 1X Tris Borate EDTA (TBE) (Wisent, Canada). Ethidium bromide stained DNA amplicons were visualized using a gel imaging system (Wealtec, Dolphin-View, NV, USA). To determine the expected bp lengths, DNA marker with defined molecular weights in the range 100-3000 bp were used.

For the presence of *VanA, VanB, Van C* genes, 1030, 433, 796 bp genes products, for *aac(6')-1e-aph(2'')-1a, aph(2'')-1b, aph(2'')-1c, aph(2'')-1d* genes, 505, 906, 627, 642 bp genes products were evaluated respectively (Figure 1).

Statistical analysis

Data were analyzed using Statistical Package for Social Sciences. Comparison for categorical variables was calculated using chi-square test. A P-value <0.05 was considered statistically significant.

Results

In this study, it was determined that *Enterococcus* strains isolated from the samples most common submitted from Internal Medicine (13%), Medical Intensive Care (11%), Surgical Intensive Care Unit (10%), pediatrics (10%),

Table 1. Primer sequences used in polymerase chain reaction methods.

Genes	Primers (5′→3′)	Product, base pairs
Van A	CAT GAA TAG AAT AAA AGT TGC AAT A CCC CTT TAA CGC TAA TAC GAT CAA	1030
Van B	GTG ACA AAC CGG AGG CGA GGA CCG CCA TCC TCC TGC AAA AAA	433
Van C	GAA AGA CAA CAG GAA GAC CGC ATC GCA TCA CAA GCA CCA ATC	796
Aac(6')-1e-aph(2'')-1a	GAGCAATAAGGGCATACCAAAAATC CCGTGCATTTGTCTTAAAAAACTGG	505
Aph(2'')-1b	TATGGATTCATGGTTAACTTGGACGCTGAG ATTAAGCTTCCTGCTAAAATATAAACATCTCTGCT	906
Aph(2'')-1c	GAAGTGATGGAAATCCCTTCGTG GCTCTAACCCTTCAGAAATCCAGTC	627
Aph(2'')-1d	GGTGGTTTTTACAGGAATGCCATC CCCTCTTCATACCAATCCATATAACC	642

Table 2. The steps and temperatures in polymerase chain reaction amplifications.

Genes	Denaturation	Annealing	DNA chain extension	Final extension
VanA, VanB, VanC	94°C (30 s)	58°C (30 s)	72°C (30 s)	72°C (10 min)
aac(6')-1e-aph(2'')-1a	94°C (1 min)	61°C (1 min)	72°C (1 min)	72°C (10 min)
aph(2'')-1b	94°C (1 min)	55°C (1 min)	72°C (1 min)	72°C (10 min)
aph(2'')-1c	94°C (1 min)	55°C (1 min)	72°C (1 min)	72°C (10 min)
aph(2'')-1d	94°C (1 min)	53.4°C (1 min)	72°C (1 min)	72°C (10 min)

Infectious Disease (9%) and Urology (9%) clinics. It was found that 52% of the strains isolated from the urine, 30% from wound, 14% from blood, 2% from abscess, 2% of them were isolated from the peritoneal fluid samples. And 58 of the strains were *E. faecalis*, 38 of them were *E. faecium* and one of them was *E. gallinorum*. There was no beta lactamase production in these strains. The antibiotics to which the strains were the most susceptible in this study were teicoplanin (94%), linezolid (91%), vancomycin (90%), ampicillin (70%), penicillin (70%), nitrofurantoin (65%), levofloxacin (41%), tetracycline (36%), erythromycin (17%). High-level gentamicin resistance (HLGR) was detected in 40% of strains, high-level streptomycin resistance (HLSR) was detected in 60% of strains. *E. faecium* strains were found more resistant to nitrofurantoin, ampicillin and penicillin and more susceptible to tetracycline than the other strains (P<0.001). There was no difference between HLSR and HLGR in different species (P>0.05). On the other hand *E faecalis* strains were found more susceptible to erytromycin (0.031), nitrofurantoin (P<0.001), levofloxacin (P=0.035), linezolid (P=0.028), teicoplanin (P=0.003), ampicillin (P<0.01), penicillin (P<0.01), HLS (P=0.02) but more resistant to tetracycline (P<0.001) than the other strains. MIC range of vancomycin was found from 0,5 to 256 µg/mL by GDM. And MIC_{50} and MIC_{90} value of vancomycin were found 1.5 and 3 µg/mL respectively. MIC_{50} and MIC_{90} values were in the range of sensivity limits. Ten strains were found resistant to vancomycin by automated system. Eight of these strains were *E. faecalis* and two of them were *E. faecium*. The rate of vancomycin susceptibilities of *Enterococcus* spp. determined by automated system in this study is shown in Table 3.

The five strains were determined resistant to vancomycin by GDM. All of these strains were *E. faecium*. The rate of vancomycin susceptibilities of *Enterococcus* spp. determined

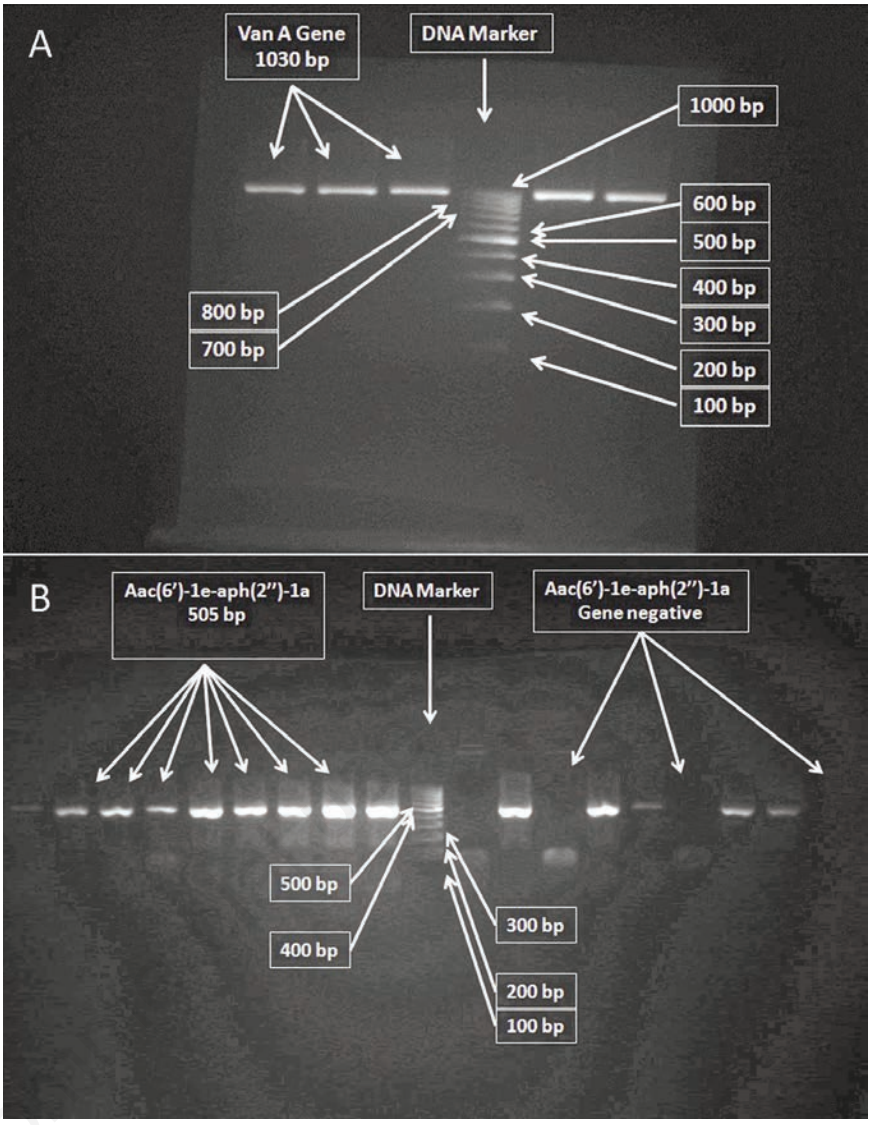

Figure 1. Polymerase chain reaction amplification products of the *vanA* gene (A) and the *aac(6')-1e-aph(2'')-1a gene* (B).

Table 3. Vancomycin susceptibility of *Enterococcus* species determined by the automated system.

Enterococcus species	Vancomycin susceptibility		Total	P
	Susceptible, N (%)	Resistant, N (%)		
E. faecalis	56 (96.5)	2 (3.5)	58	>0.05
E. faecium	30 (78.9)	8 (21.1)	38	0.006
Other	4 (100)	0 (0)	4	>0.05
Total	90 (90)	10 (10)	100	

Table 4. Vancomycin susceptibility status of *Enterococcus* species determined by gradient difusion method.

Enterococcus species	Vancomycin susceptibility		Total	P
	Susceptible, N (%)	Resistant, N (%)		
E. faecalis	58 (100)	0 (0)	58	>0.05
E. faecium	33 (86.4)	5 (13.6)	38	0.007
Other	4 (100)	0 (0)	4	>0.05
Total	95 (95)	5 (5)	100	

by GDM in this study is shown in Table 4.

VanA gene was detected in five strains. Gel image of the five strains containing *VanA* gene is shown in Figure 1A. *VanB* and *Van C* genes were detected in none of the strains (Table 5).

All of the strains carrying *VanA* gene were *E. faecium*. All of the strains carrying *VanA* gene were found to be resistant to vancomycin, nitrofurantoin, teicoplanin, ampicillin and penicillin (P<0.001). MIC values of vancomycin and MIC values of teicoplanin in five VanA positive strains were found higher than MIC values in 95 VanA negative strains (P<0.001).

aac (6 ')-1-aph (2'')-1 gene was detected in 58 strains. *aph (2'')-1b, aph (2'')-1c* and *aph (2'')-1d* genes were not detected in the strains. And 4 strains were detected to carry both *aac(6')-1e-aph(2'')-1a* gene and *VanA* gene. HLGR and HLSR were found more frequently in strains carrying *aac(6')-1e-aph(2'')-1a* gene (P<0.001) (Table 6).

VanA gene was found in five (5%) *E. faecium*. *aac (6 ')-1e-aph (2'')-1a* gene was detected in 22 (38%) *E. faecium*.

Discussion and Conclusions

Enterococci are situated between troubled bacteria because of observed in the increasing rate in nosocomial infections and carrying clindamycin, fluoroquinolones, trimethoprim-sulfamethoxazole, low level penicillin, low-level aminoglycoside and transferring of genetic material or acquired tetracycline, erythromycin, rifampin, chloramphenicol, nitrofurantoin, fusidic acid, and beta-lactam HLAR, fluoroquinolones and vancomycin resistance by mutation.[4]

Initially, the beta-lactamase-producing strains of enterococci were reported as rare in the United States but nowadays isolates all over the world.[4] In our study, no beta lactamase production was determined. Many studies conducted in our country, beta-lactamase-producing strain did not appear as similar to our study.[11,12] Beta-lactamase production in enterococci has not been reported in many studies conducted in foreign countries as similar to studies in our country.[13,14]

Partial or complete beta-lactam resistance is characteristic in Enterococcus species. *E. faecalis* is 10-100 times less susceptible to penicillin than other streptococcal species. *E. faecium* is 4-16 times less susceptible to penicillin than *E. faecalis*.[4] In our study, it was determined 30 (30%) of the strains are resistant to ampicillin and penicillin. The strains of *E. faecalis* were more susceptible to ampicillin and penicillin. In our country, Kacmaz and colleagues found that penicillin and ampicillin resistance rates of all strains were respectively 27 and 26%.[11] In other countries, D'azevedo and colleagues determined that only 14 strains were high-level ampicillin resistant in their study with 455 enterococci.[15] In our study, nitofurantoin resistance rate was 35%. In other countries, Moaddab and colleagues found that nitrofurantoin resistance was 1,5%, Akhtar and colleagues found 5%.[13,16]

In our study, tetracycline resistance has been identified in 64% of strains. The tetracycline resistance rate has been reported as 8.3, 51% and 70.4% in some studies.[17-19]

In our study, erythromycin resistance was found to be 69%. Sirin and Adiloglu detected that erythromycin resistance rate was 38%.[18] Comert and colleagues have found that all of six VRE strains were resistant to erythromycin in their study.[20] Kirdar and colleagues found that all of 12 VRE strains were resistant to erythromycin in their study.[19]

Oxazolidinone's are new member of the synthetic antibiotics group with activity against Gram-positive. In our study, linezolid resistance was found to be 7%. Ak and colleagues found that resistance rate for *E. faecalis* was 10.2%, for *E. faecium* was 9.1% in their study.[21] These rates were lower in other countries. Akhter and colleagues detected that linezolid resistance rate was 4% in their study.[16] Protonotario and colleagues detected that linezolid resistance rate for *E. faecalis* and *E. faecium* were respectively 0.3%, 1.6% in their study.[22]

Enterococcus infections carrying resistance genes against glycopeptides, penicillin and aminoglycoside group of antibiotics lead to serious problems. The number of enterococci strains which are resistant glycopeptide antibiotics such as vancomycin and teicoplanin gradually increase.[2] Glycopeptide antibiotic resistance in enterococci group was first reported in 1988.[6] Then high-level of vancomycin-and teicoplanin-resistant strains has spread all over the world. Efe and colleagues have isolated 21 (18.8%) VRE strains in 112 patients.[23] Sirin and Adiloglu determined that all strains were susceptible to vancomycin and teicoplanin except one strain moderately susceptible to vancomycin in 100 enterococci strains in their study.[18] Ak and colleagues detected five vancomycin-resistant, one moderately vancomycin resistant strains with automated systems in their study.[21]

In our study, ten vancomycin resistant strains were detected by automated systems, but just five vancomycin resistant strains were identified by GDM. In our country, Karaca and colleagues did not find vancomycin resistance in enterococci by GDM.[24] Protonotario and colleagues found vancomycin resistance rate of 0.5% for *E. faecalis*, 9.6 % for *E. faecium* by automated systems in their studies containing 1498 *E. faecalis* and 625 *E. faecium* strains.[22] Zouain and colleagues examined vancomycin and teicoplanin susceptibility of 153 enterococci strains by the disk diffusion and GDM method. The vancomycin or teicoplanin resistance was determined in only one *E. gallinarum* strain, which confers moderate resistance against vancomycin.[25] Hallgren and colleagues

Table 5. The number of strains containing *VanA, VanB, VanC* gene.

Genes	Positive, N (%)	Negative, N (%)	Total
Van A	5 (5)	95 (95)	100
Van B	0 (0)	100 (100)	100
Van C	0	100 (100)	100

Table 6. High-level gentamicin resistance (HLGR) and high-level streptomycin resistance (HLSR) rates of strains containing a*ac(6')-1e-aph(2'')-1a* gene.

Antibiotics	Susceptibility status	*aac(6')-1e-aph(2'')-1a* gene		Total	P
		Positive, N (%)	Negative, N (%)		
HLSR	R	47 (74.6)	16 (25.4)	63 (100)	<0.001
	S	11 (29.7)	26 (70.3)	37 (100)	
HLGR	R	40 (100)	0 (0)	40 (100)	<0.001
	S	18 (30)	42 (70)	60 (100)	

R, resistant; S, susceptible.

determined vancomycin resistance in rate of 3.9% by GDM in Sweden, Paberzo and colleagues determined 20% by GDM in Lithuania, Udo and colleagues determined 3% by GDM in Kuwait.[26-28]

VanA and VanB are the most common resistance genotypes, although there are seven different glycopeptide resistant genotypes found in enterococci. In United States and Europe VanA resistance phenotype is more common than others.[29,30] When compared with other types of enterococci, VanA resistance phenotype occurs more frequently. In our study, VanA type resistance have been identified in five of strains by PCR. All of these five strains were *E. faecium*. First time in Turkey VanA phenotypes of *E. faecium* was isolated in 2001.[31] Then, in 2002, outbreak of VanA phenotype *E. faecium* was reported.[32] Coskun and colleagues identified *VanA* and *VanB* genes in thirty and five strains respectively.[33] These are the first VanB positive *E. faecium* strains in our country. And also Kirdar and colleagues also found *VanA* gene in 12 vancomycin resistant *E. faecium* isolated from patients with hematologic malignancies.[19] In a study in 2000 with the participation of our country, the prevalence of vanA VRE was found highest in the United Kingdom (2.7%), while the prevalence of vanB VRE was highest in Slovenia (2%).[34] The prevalence of VanC VRE was highest in Latvia and Turkey, where rates were 14.3 and 11.7%, respectively. The highest prevalence of high-level gentamicin-resistant enterococci was seen in Turkey and Greece.[34]

Contrast to VanA type, which is common worldwide, VanB type is much less frequently encountered but VanB type have been reported from different countries.[35] Nelson and colleagues found that seven *E. faecalis* strains have VanA phenotype in their study, which includes vancomycin resistant 144 (93.5%) *E. faecium*, seven (4.5%) *E. faecalis* and three (2%) *E. gallinarum.*[36] It was determined that remaining six strains have VanA phenotype and 138 of 144 *E. faecium* have VanB phenotype. *Van C-1* gene was amplified in one of three *E. gallinarum* strains. Descheemaeker and colleagues reported that 46.1% of 601 vancomycin resistant strains was carrying *VanA* gene while enterococci carrying *VanB* gene were not detected.[30]

Low-level aminoglycoside resistance in enterococci, due to a reduction of the permeability of the cell wall. The high-level resistance is mediated by ribosomal or inactivating enzymes. A synergistic beta-lactam-aminoglycoside bactericidal effect is eliminated in the presence of high-level aminoglycoside resistance. High-level aminoglycoside-resistant enterococci are important because they can be resistant to other antibiotics. In our study HLGR rate was 40%, HLSR rate was determined as 63%. No difference was found between *Enterococcus* species according to HLGR and HLSR rates. High-level aminoglycoside resistance rate was repoted as 16% in the study from our country.[11] Sirin and Adiloglu also reported HLGR and HLSR rate as 23 and 16% respectively.[19] Another two studies HLAR rates were reported as 39.7 and 54.5% in *E. faecalis*, 9.1 and 36.3% in *E. faecium.*[11,18] HLAR rate was reported 48.1% in Turkey by European vancomycin-resistant enterococci study group.[34] HLGR rate was reported in a range 5-65%, HLSR rate was reported in a range 14-50.4% in the studies from other contries.[13,14,37-39]

The addition of aminoglycoside to an antibiotic, which inhibits cell wall synthesis, plays a significant role in increasing effect of both drugs. However acquired genes through plasmid and transposon lead to release aminoglycosides modifying enzymes and high level aminoglycosides resistance. Thus, the combination treatment loses its synergistic effect.[4,5] The most common aminoglycoside modifying enzymes in enterococci is APH (2'')-AAC (6 ') enzyme, which is consisting of two enzymes and responsible for resistance to all aminoglycosides except streptomycin.[4] *aac(6')-Ie-aph(2'')-Ia* gene, which encoding bifunctional aac (6')-Ie-aph (2'')-Ia enzyme is the most common clinically.

Aminoglycoside-modifying enzymes are the responsible from high-level streptomycin resistance.[5,6] Aminoglycoside-modifying enzyme encoding aminoglycoside resistance genes found so far in Enterococcus are *aac(6')-Ie-aph(2'')-Ia, aph(2'')-Ib, aph(2'')-Ic, aph(2'')-Id, aph(3')-IIIa, aac(6')-Ii, ant(3'')-Ia, ant(4')-Ia, ant(6')-Ia*.[5-7] In our study, *aac (6')-Ie-aph (2'')-Ia* gene was identified in 58 (58%) strains. In no strains *aph (2'')-Ib, aph (2'')-Ic* and *aph (2'')-Id* genes were detected. Feizabadi and colleagues found that *aac(6')-Ie-aph(2'')-Ia* gene was detected in 59 strains of 114 enteroocci strains and *aph (2'')-Ic* gene was detected in 2 *E. faecium* strains by PCR in their study.[37] Similar to our study, none of *aph (2'')-Ib* and *aph (2'')-Id* gene were not detected. In a study conducted by 279 enterococci strains obtained at a university hospital in Japan, *aac (6 ')-Ie-aph (2'')-Ib* gene was found to be more than *E. faecium* (4.3%) compared with *E. faecalis* (42.5%). *aph (2'')-Ic* gene was not detected in the strains of any enterococci.[40]

In recent years, the emergence of enterococci strains, which cause increasing frequency infections, has significantly restricted antibiotics used to treat infections. Glycopeptides are the most effective agents against enterococci. However, the unnecessary utilization will result increasing the frequency of glycopeptide-resistant enterococci and treatment impasse. Another problem encountered in the treatment of enterococcal infections is HLAR. Treatment options are limited in high-level aminoglycoside-resistant enterococci because of disappeared beta-lactam-aminoglycoside combination for synergistic bactericidal effect. For these reasons, accurate identification of enterococci, determination of antimicrobial resistance state in time, a different resistance pattern and to reveal the resistance mechanisms is important.

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