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HIGH PREVALENCE OF VANCOMYCIN AND HIGH-LEVEL GENTAMICIN RESISTANCE IN *ENTEROCOCCUS FAECALIS* ISOLATES

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Multiple drug-resistant enterococci are major cause of healthcare-associated infections due to their antibiotic resistance traits. Among them, *Enterococcus faecalis* is an important opportunistic pathogen causing various hospital-acquired infections. A total of 53 *E. faecalis* isolates were obtained from various infections. They were identified by phenotypic and genotypic methods. Determination of antimicrobial resistance patterns was done according to CLSI guidelines. The isolates that were non-susceptible to at least one agent in ≥3 antimicrobial categories were defined as multidrug-resistant (MDR). Detection of antimicrobial resistance genes was performed using standard procedures. According to MDR definition, all of the isolates were MDR (100%). High-level gentamicin resistance was observed among 50.9% of them (MIC ≥ 500 µg/ml). The distributions of *aac(6′)-Ie-aph(2′′)-Ia* and *aph(3′)-IIIa* genes were 47.2% and 69.8%, respectively. The *aph(2′′)-Ib, aph(2′′)-Ic, aph(2′′)-Id*, and *ant(4′)-Ia* genes were not detected. Vancomycin resistance was found in 45.3% of strains. The *vanA* gene was detected in 37.7% of isolates, whereas *vanB* and *vanC*$_1$ genes were not observed in any strain. Erythromycin resistance rate was 79.2% and the frequencies of *ermB* and *ermC* genes were 88.6% and 69.8%, respectively. The *ermA* and *msrA* genes were not present in any of the isolates. Our data indicate a high rate of MDR *E. faecalis* strains. All of high-level gentamicin-resistant isolates carried at least one of *aac(6′)-Ie-aph(2′′)-Ia* or *aph(3′)-IIIa* genes. Distribution of *vanA* was notable among the isolates. In addition, *ermB* and *ermC* were accountable for resistance to erythromycin.

Keywords: multidrug-resistant, high-level gentamicin-resistant, vancomycin-resistant enterococci, *Enterococcus faecalis*, Iran

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Introduction

Multiple drug-resistant enterococci are the major cause of healthcare-associated infections due to their virulence and antibiotic resistance traits [1–4]. They are considered as critical agent for the dissemination of antimicrobial resistance determinants to other bacteria through mobile genetic elements [2, 5].

High-level aminoglycoside resistance in enterococci is usually mediated by aminoglycoside-modifying enzymes, including acetyltransferase, phosphotransferase, and nucleotidyltransferase [6]. They catalyze the covalent modification of amino and hydroxyl groups within the aminoglycosides [7]. High-level gentamicin-resistant [HLGR; minimum inhibitory concentration (MIC) ≥ 500 μg/ml] enterococci harbor *aac(6′)-Ie-aph(2′′)-Ia* gene that encodes a bifunctional aminoglycoside-modifying enzyme [AAC(6′)-Ie-APH(2′′)-Ia]. This enzyme is associated with resistance to all available aminoglycosides except streptomycin [6, 7]. Moreover, three aminoglycoside resistance genes, such as *aph(2′′)-Ib, aph(2′′)-Ic*, and *aph(2′′)-Id*, have been recognized among gentamicin-resistant strains. The *aph(3′)-IIIa* and *ant(4′)-Ia* genes also encode resistance to various aminoglycosides [7, 8].

Vancomycin-resistant enterococci (VRE)-associated infections are more severe than infections with vancomycin-susceptible enterococci [9]. Resistance to vancomycin is mediated by *van* gene clusters. VRE harbors the transmissible *vanA* or *vanB* genetic elements that encode high-level resistance to vancomycin [10, 11]. Low-level vancomycin resistance is also related to *vanC* gene [12]. Prolonged hospitalization, prior exposure to antibiotics, and use of foreign medical devices, such as catheters, are risk factors for VRE acquisition [10, 13].

Due to general usage of macrolides, resistance to this family has increased among enterococci [14]. Two major mechanisms including target modification by the ribosomal methylase encoded by *erm* genes and efflux pump systems encoded by *msr* and *mefA/E* genes mediate macrolide resistance [15]. The *erm* gene carried by enterococci results in either inducible or constitutive resistance to all macrolides, lincosamides, and streptogramin B. On the other hand, the *msrA* gene confers inducible resistance to streptogramin B and some macrolide antibiotics [16].

Among the enterococci, *Enterococcus faecalis* is an important opportunistic pathogen causing various hospital-acquired infections [14, 17]. However, since there is limited data regarding the molecular characteristics of multidrug-resistant (MDR) *E. faecalis* isolates in hospitalized patients in Iran, this study was designed to examine the prevalence of genes encoding antimicrobial resistance among *E. faecalis* strains isolated from hospitalized patients in Shiraz, south west of Iran.

Materials and Methods

Bacterial isolates

A total of 53 *E. faecalis* isolates were obtained from infections in Nemazee Hospital (the main hospital affiliated to Shiraz University of Medical Sciences) from September 2015 to March 2016 according to definitions of nosocomial infections [18]. They were obtained from the urinary tract infections ($n = 40$), respiratory tract infections (pleural fluid and endotracheal tube; $n = 5$), bloodstream infections ($n = 4$), abdominal infections ($n = 2$), eye infection ($n = 1$), and transjugular intrahepatic portosystemic shunt ($n = 1$). Only one isolate per patient was included. This study was approved by the ethics committee of Shiraz University of Medical Sciences (Register code: IR.SUMS.REC.1397.090). Enterococcal isolates were identified according to conventional microbiological tests, such as Gram staining (Gram positive), catalase reaction (catalase negative), growth on brain–heart infusion (BHI) agar (Conda, Madrid, Spain) with 6.5% NaCl, and bile-esculin test (positive) [19]. The *ddlE* gene was amplified by polymerase chain reaction (PCR) using *E. faecalis* specific primers (*ddlE. faecalis* F-5′- ATCAAGTACAGT-TAGTCT-3′ and R-5′-ACGATTCAAAGCTAACTG-3′) for molecular confirmation [12]. The PCR procedure consisted of a pre-denaturation step at 95 °C for 5 min, followed by 30 cycles for 60 s at 95 °C, 45 s at 45 °C, and 50 s at 72 °C. A final extension step was performed at 72 °C for 5 min.

Determination of MDR and HLGR isolates

Antimicrobial susceptibility tests were performed using disc diffusion method on the Mueller–Hinton Agar (Merck Co., Germany) based on Clinical and Laboratory Standards Institute (CLSI) guideline [20]. The tested antibiotics (Mast Group Ltd., UK) were vancomycin (30 μg), teicoplanin (30 μg), erythromycin (15 μg), penicillin (10 units), ampicillin (10 μg), ciprofloxacin (5 μg), tetracycline (30 μg), fosfomycin (200 μg), nitrofurantoin (300 μg), rifampin (5 μg), quinupristin–dalfopristin (15 μg), and linezolid (5 μg). High-level gentamicin resistance was also determined by the broth microdilution method using BHI broth (Conda) according to CLSI guideline [20]. *Staphylococcus aureus* ATCC 25923 and *E. faecalis* ATCC 29212 were used as the standard strains.

Multidrug resistance was determined according to definitions of MDR bacteria [21]. MDR definition for *Enterococcus* spp. is the isolate that were non-susceptible (including resistant or intermediate) to at least one agent in ≥3 antimicrobial categories [21].

DNA extraction and detection of resistance genes

Genomic DNA was extracted from fresh grown colonies, as described previously [22]. PCR was performed to detect the genes encoding resistance to aminoglycoside, glycopeptide, and macrolide antibiotics [*aac(6′)-Ie-aph(2′′)-Ia, aph(2′′)-Ib, aph(2′′)-Ic, aph(2′′)-Id, aph(3′)-IIIa, ant(4′)-Ia, vanA, vanB, vanC$_1$, ermA, ermB, ermC*, and *msrA*] among strains [8, 12, 23]. The products were separated by electrophoresis in 1% agarose gels with 1X Tris/acetate/EDTA buffer, stained with safe stain load dye (CinnaGen Co., Iran) and visualized under ultraviolet illumination.

Statistical analysis

The distribution of aminoglycoside resistance genes among HLGR and non-HLGR isolates was calculated by χ^2 and Fisher's exact tests for each gene. The prevalence of glycopeptide resistance genes among glycopeptide non-susceptible and susceptible isolates was calculated by the aforementioned tests, and the presence of macrolide resistance genes among macrolide non-susceptible and susceptible isolates was also calculated. A p value of ≤ 0.05 was considered as statistically significant.

Results

According to MDR definition, all of 53 *E. faecalis* strains were MDR (100%). The antibiotic resistance patterns are shown in Table I. High-level gentamicin resistance (MIC $\geq$ 500 µg/ml) was observed among 27 (50.9%) isolates. The distributions of *aac(6′)-Ie-aph(2′′)-Ia* and *aph(3′)-IIIa* genes were 25 (47.2%) and 37 (69.8%), respectively (Table I). The *aph(2′′)-Ib, aph(2′′)-Ic, aph (2′′)-Id*, and *ant(4′)-Ia* resistance genes were not detected in any of the isolates. The prevalence of *aac(6′)-Ie-aph(2′′)-Ia* gene among HLGR isolates was more than non-HLGR isolates, significantly ($p = 0.019$). In addition, the *aph(3′)-IIIa* gene was more frequent in HLGR in comparison to non-HLGR isolates with a significant correlation ($p < 0.001$). The distribution of aminoglycoside resistance genes among HLGR and non-HLGR isolates is shown in Table II.

Vancomycin resistance was found among 24 (45.3%) strains, whereas the *vanA* gene was found in 20 (37.7%) isolates (Table I). All strains were negative for *vanB* and *vanC$_1$* genes amplification.

Resistance to erythromycin was detected in 42 (79.2%) of isolates, and the frequencies of *ermB* and *ermC* genes were 47 (88.6%) and 37 (69.8%),

Table I. The distribution of resistance genes among MDR *Enterococcus faecalis* isolates

No. of isolates	Infections (*N*)	Resistance patterns	MIC of GM (μg/ml)	Resistance genes
1	Urinary tract infection (40)	VAN, RIF, ERY, TET, and QDA	>2,048	*vanA, ermB, aac(6′)-Ie-aph (2′′)-Ia*, and *aph(3′)-IIIa*
2		RIF, ERY, TET, and QDA	256	*ermB, ermC*, and *aac(6′)-Ie-aph(2′′)-Ia*
3		RIF, ERY, TET, CIP, and QDA	128	*ermB* and *aph(3′)-IIIa*
4		VAN, RIF, TET, and QDA	1,024	*vanA, ermB, ermC*, and *aph (3′)-IIIa*
5		VAN, RIF, ERY, TET, QDA, and AMP	2,048	*vanA, ermB, ermC*, and *aph (3′)-IIIa*
6		VAN, RIF, ERY, TET, and QDA	1,024	*ermB, ermC*, and *aph(3′)-IIIa*
7		TET and QDA	128	*vanA, ermB, ermC*, and *aac (6′)-Ie-aph(2′′)-Ia*
8		VAN, TET, and QDA	16	–
9		VAN, ERY, TET, CIP, QDA, TEC, and AMP	>2,048	*ermB, aac(6′)-Ie-aph(2′′)-Ia*, and *aph(3′)-IIIa*
10		ERY, TET, CIP, and QDA	128	*ermB, ermC*, and *aac(6′)-Ie-aph(2′′)-Ia*
11		VAN, ERY, TET, CIP, and QDA	>2,048	*ermB, aac(6′)-Ie-aph(2′′)-Ia*, and *aph(3′)-IIIa*
12		VAN, RIF, ERY, TET, CIP, and QDA	64	*ermB* and *aph(3′)-IIIa*
13		RIF, ERY, TET, and QDA	>2,048	*ermB, ermC, aac(6′)-Ie-aph (2′′)-Ia*, and *aph(3′)-IIIa*
14		VAN, TET, QDA, and AMP	2,048	*vanA, ermB, ermC*, and *aph (3′)-IIIa*
15		VAN, NIT, RIF, ERY, TET, CIP, PEN, QDA, TEC, and AMP	256	*vanA, ermB, ermC, aac(6′)-Ie-aph(2′′)-Ia*, and *aph(3′)-IIIa*
16		RIF, TET, and QDA	256	*aac(6′)-Ie-aph(2′′)-Ia*, and *aph (3′)-IIIa*
17		RIF, ERY, TET, CIP, PEN, and AMP	128	*vanA, ermB*, and *aph(3′)-IIIa*
18		VAN, NIT, ERY, CIP, PEN, and AMP	512	*ermB* and *aph(3′)-IIIa*
19		RIF, ERY, TET, CIP, PEN, and AMP	>2,048	*vanA, ermB, aac(6′)-Ie-aph (2′′)-Ia*, and *aph(3′)-IIIa*
20		RIF and TET	16	*ermB* and *ermC*
21		ERY, TET, and QDA	16	*ermB*
22		VAN	512	*vanA, ermC*, and *aac(6′)-Ie-aph(2′′)-Ia*
23		ERY, TET, and QDA	32	*ermB*
24		RIF, ERY, TET, and QDA	8	*vanA, ermB*, and *ermC*

Table I. (cont.)

No. of isolates	Infections (*N*)	Resistance patterns	MIC of GM (μg/ml)	Resistance genes
25		ERY, TET, CIP, and QDA	>2,048	*ermB, ermC, aac(6′)-Ie-aph (2′′)-Ia*, and *aph(3′)-IIIa*
26		VAN, RIF, ERY, TET, CIP, PEN, QDA, TEC, and AMP	512	*vanA, ermB, ermC*, and *aph (3′)-IIIa*
27		VAN, NIT, ERY, CIP, PEN, TEC, and AMP	>2,048	*ermB, ermC, aac(6′)-Ie-aph (2′′)-Ia*, and *aph(3′)-IIIa*
28		VAN, RIF, ERY, TET, CIP, PEN, QDA, and AMP	2,048	*vanA, ermB, ermC, aac(6′)-Ie-aph(2′′)-Ia*, and *aph(3′)-IIIa*
29		TET and QDA	16	*ermB* and *ermC*
30		VAN, RIF, ERY, TET, CIP, PEN, TEC, and AMP	512	*ermB, ermC*, and *aph(3′)-IIIa*
31		ERY, TET, CIP, PEN, and AMP	>2,048	*ermB, ermC, aac(6′)-Ie-aph (2′′)-Ia*, and *aph(3′)-IIIa*
32		NIT, RIF, ERY, TET, CIP, PEN, and AMP	2,048	*ermB, ermC, aac(6′)-Ie-aph (2′′)-Ia*, and *aph(3′)-IIIa*
33		ERY, TET, CIP, and QDA	>2,048	*vanA, ermB, ermC*, and *aac (6′)-Ie-aph(2′′)-Ia*
34		ERY, TET, and QDA	256	*ermB, ermC*, and *aph(3′)-IIIa*
35		RIF, ERY, TET, and QDA	256	*ermB, ermC*, and *aph(3′)-IIIa*
36		ERY, TET, CIP, PEN, and QDA	512	*ermB, ermC, aac(6′)-Ie-aph (2′′)-Ia*, and *aph(3′)-IIIa*
37		ERY, TET, CIP, and QDA	2,048	*ermB, ermC, aac(6′)-Ie-aph (2′′)-Ia*, and *aph(3′)-IIIa*
38		TET and QDA	4	*ermC*
39		VAN, RIF, ERY, TET, CIP, PEN, TEC, and AMP	128	*vanA, ermB, aac(6′)-Ie-aph (2′′)-Ia*, and *aph(3′)-IIIa*
40		TET, PEN, and QDA	32	–
41	Respiratory tract infection (5)	VAN, ERY, TET, and QDA	128	*vanA, ermB, aac(6′)-Ie-aph (2′′)-Ia*, and *aph(3′)-IIIa*
42		VAN, RIF, ERY, TET, CIP, PEN, TEC, and AMP	512	*vanA, ermB, ermC*, and *aph (3′)-IIIa*
43		ERY, TET, and QDA	>2,048	*vanA, ermB, ermC, aac(6′)-Ie-aph(2′′)-Ia*, and *aph(3′)-IIIa*
44		ERY, TET, CIP, PEN, TEC, and AMP	128	*vanA, ermB, ermC*, and *aph (3′)-IIIa*
45		VAN, ERY, TET, CIP, and QDA	256	*ermB* and *aph(3′)-IIIa*
46	Bloodstream infection (4)	ERY, TET, and QDA	256	*ermB, ermC, aac(6′)-Ie-aph (2′′)-Ia*, and *aph(3′)-IIIa*

Table I. (cont.)

No. of isolates	Infections (*N*)	Resistance patterns	MIC of GM (μg/ml)	Resistance genes
47		NIT, ERY, TET, QDA, and AMP	16	*ermC*
48		ERY, TET, CIP, PEN, QDA, and AMP	32	*ermB* and *ermC*
49		VAN, RIF, ERY, TET, CIP, PEN, TEC, and AMP	2,048	*ermB, ermC, aac(6′)-Ie-aph (2′′)-Ia*, and *aph(3′)-IIIa*
50	Abdominal infection (2)	VAN, ERY, TET, and QDA	>2,048	*vanA, ermB, ermC, aac(6′)-Ie-aph(2′′)-Ia*, and *aph(3′)-IIIa*
51		VAN, ERY, TET, and QDA	8	*ermB* and *ermC*
52	Eye infection (1)	ERY, TET, and QDA	1,024	*ermB, ermC*, and *aph(3′)-IIIa*
53	TIPS (1)	VAN	1,024	*vanA, ermB, ermC*, and *aph(3′)-IIIa*

Note: VAN: vancomycin; NIT: nitrofurantoin; RIF: rifampin; ERY: erythromycin; TET: tetracycline; CIP: ciprofloxacin; PEN: penicillin; QDA: quinupristin–dalfopristin; TEC: teicoplanin; AMP: ampicillin; GM: gentamicin; MIC: minimum inhibitory concentration; TIPS: transjugular intrahepatic portosystemic shunt.

respectively (Table I). However, the *ermA* and *msrA* genes were not detected in any of the isolates.

The distribution of resistance genes among non-susceptible and susceptible isolates to related antibiotics is shown in Tables III and IV.

Discussion

E. faecalis is a common pathogen that can cause severe hospital-acquired infections [17, 24]. In this study, all strains isolated from various infections were MDR. Although this finding was more than any other studies in Iran [25–27], high rates of multidrug resistance among *E. faecalis* strains were also reported from other countries [15, 28].

Our results indicated that 50.9% of isolates were HLGR (MIC ≥ 500 μg/ml). In the several studies, high-level gentamicin resistance among *E. faecalis* isolates was reported in 65%–96% of the strains [29–31].

In this study, the most prevalent aminoglycoside resistance gene among the HLGR isolates was *aph(3′)-IIIa* (92.6%), and the *aac(6′)-Ie-aph(2′′)-Ia* gene was found in 62.9% of them (Table II). It was contrary to previous studies that the *aac (6′)-Ie-aph(2′′)-Ia* gene has been characterized as the main aminoglycoside

Table II. Distribution of *aac(6′)-Ie-aph(2′′)-Ia* and *aph(3′)-IIIa* genes among HLGR and non-HLGR isolates

Isolates (*N*)	Resistance genes	
	aac(6′)-Ie-aph(2′′)-Ia [*N* (%)]	*aph(3′)-IIIa* [*N* (%)]
HLGR (27)	17 (62.9)	25 (92.6)
Non-HLGR (26)	8 (30.7)	12 (46.1)
Total (53)	25 (47.2)	37 (69.8)

Note: HLGR: high-level gentamicin-resistant.

Table III. Distribution of *vanA* gene among glycopeptide non-susceptible and susceptible isolates

Isolates (*N*)	*vanA* gene [*N* (%)]
Glycopeptide non-susceptible (43)	18 (41.8)
Glycopeptide susceptible (10)	2 (20.0)
Total (53)	20 (37.7)

Table IV. Distribution of *ermB* and *ermC* genes among macrolide non-susceptible and susceptible isolates

Isolates (*N*)	Resistance genes	
	ermB [*N* (%)]	*ermC* [*N* (%)]
Macrolide non-susceptible (51)	46 (90.1)	35 (68.6)
Macrolide susceptible (2)	1 (50.0)	2 (100.0)
Total (53)	47 (88.6)	37 (69.8)

resistance gene [30–32]. In a study conducted in Tehran, north of Iran, the *aac(6′)-Ie-aph(2′′)-Ia* and *ant(4′)-Ia* genes were detected in HLGR enterococci [30]. It seems that *aph(3′)-IIIa* gene is the predominant related gene in our region. It is often carried on a conjugative plasmid among enterococci [33] and therefore the isolates could acquire it by conjugation.

We found that all of the HLGR isolates carried at least one of *aac(6′)-Ie-aph (2′′)-Ia* or *aph(3′)-IIIa* genes (Table I). However, 30.7% and 46.1% of non-HLGR isolates harbored *aac(6′)-Ie-aph(2′′)-Ia* and *aph(3′)-IIIa* genes, respectively (Table II). This may be due to downregulation of genes expression.

All of the studied isolates were also negative for *aph(2′′)-Ib, aph(2′′)-Ic, aph(2′′)-Id*, and *ant(4′)-Ia* genes amplification. Absence of the *aph(2′′)-Ib, aph(2′′)-Ic*, and *aph(2′′)-Id* genes in *E. faecalis* strains was shown in several studies [34–37]. They are minor contributors to gentamicin resistance among *Enterococcus* strains [33].

The *aac(6′)-Ie-aph(2′′)-Ia* and *aph(3′)-IIIa* genes were present in 20 (37.7%) isolates, simultaneously (Table I). Co-existence of these genes among isolates of *E. faecalis* has been previously reported from Iran [34]. Co-presence of *aac(6′)-Ie-aph(2′′)-Ia* and *aph(3′)-IIIa* genes leads to failure in combination therapy by most aminoglycosides (except for streptomycin) with cell wall-active agents [6].

To the best of our knowledge, this was the first study to investigate regarding glycopeptide and macrolide resistance genes among MDR *E. faecalis* strains in Iran. In this study, more than 45% of the strains were VRE. Notable resistance to vancomycin among *E. faecalis* strains was similar to several studies in the country [38, 39]. Moreover, increasing VRE prevalence among hospitalized patients was also reported previously from southwestern Iran [40]. In contrast to our results, lower rates of VRE were reported in various studies [41–43].

We found that 37.7% of the isolates carried *vanA* gene, whereas *vanB* and $vanC_1$ genes were not detected in any of the strains. Similarly, previous researches had indicated that the *vanA* gene was the only glycopeptide resistance determinant found in *E. faecalis* isolates [2, 9, 44, 45]. According to these studies, *vanA* gene was the predominant glycopeptide resistance gene among *E. faecalis* strains. Dissemination of resistance determinants to other bacteria such as *S. aureus* using Inc18 plasmids of vancomycin-resistant *E. faecalis* strains is a serious risk of VRE colonization in hospitals [13].

Statistically, there was no significant difference between the distribution of *vanA* gene among glycopeptide non-susceptible and susceptible isolates; however, it was more prevalent in non-susceptible strains ($p > 0.05$; Table III). Susceptibility to vancomycin among *vanA*-positive isolates is probably due to deficiency in *vanA* operon. VanA protein alone cannot mediate resistance to vancomycin, and the true functions of VanH and VanR proteins are also necessary [46]. On the other hand, the *vanA* gene was not detected in some vancomycin-resistant isolates. This resistance could be due to poor penetration of antibiotic into cells or VanE-type vancomycin resistance. This type of vancomycin resistance (VanE-type) has been described in *E. faecalis* strains, which are resistant to low levels of vancomycin and susceptible to teicoplanin [46, 47].

The *vanC* genes are intrinsic property (chromosomally encoded) of *Enterococcus gallinarum, Enterococcus casseliflavus*, and *Enterococcus flavescens* [12]. This could explain absence of $vanC_1$ gene in our *E. faecalis* isolates, despite the existence of reported cases in the literature [48, 49]. This gene may have been transferred from above enterococcal species to *E. faecalis* horizontally [48].

As shown in Table I, high incidence of *ermB* and *ermC* genes was observed among analyzed strains, but the *ermA* gene was not found among

them. The various distributions of *erm* genes were shown in previous studies [5, 14, 15, 50, 51]. However, *ermB* gene is the most prevalent one among enterococcal species and *ermA* gene is more commonly found in staphylococcal strains [15, 51–56].

The *ermB* and *ermC* genes were more frequent in macrolide non-susceptible in comparison to susceptible isolates without any significant correlation ($p > 0.05$; Table IV). Suppression of gene expression may lead to erythromycin susceptibility among isolates that harbor *ermB* or *ermC* genes.

In this study, the investigated gene encoding efflux pump (*msrA*) was not found. Non-existence of *msrA* gene in *E. faecalis* strains was shown in a previous study [57]. It seems that presence and expression of *erm* genes in *E. faecalis* are sufficient for macrolide resistance.

Since all of the isolates were MDR and almost half of them were HLGR and VRE, infection control procedures are recommended to be performed in hospital. Improving hand hygiene compliance of healthcare workers is probably the most effective strategy for reducing the incidence of healthcare-associated infections. Moreover, disinfection and sterilization of medical equipment is essential for prevention and control of infection. Due to reduced biocide susceptibility in enterococci [58], constant monitoring of disinfectant agents susceptibility can be helpful to designate more effective agents.

In conclusion, we evaluated the *E. faecalis* strains isolated from various infections, and all of the strains were MDR. More than half of the isolates had MICs ≥ 500 µg/ml for gentamicin (50.9%). The distributions of *aac(6′)-Ie-aph(2′′)-Ia* and *aph(3′)-IIIa* genes were 47.2% and 69.8%, respectively. All of the studied isolates were also negative for *aph(2′′)-Ib, aph(2′′)-Ic, aph(2′′)-Id*, and *ant(4′)-Ia* genes amplification. All the HLGR isolates carried at least one of *aac(6′)-Ie-aph(2′′)-Ia* or *aph(3′)-IIIa* genes. Resistance to vancomycin was observed in more than 45% of the studied strains and erythromycin resistance was 79.2%. The *vanA* gene was found in 37.7% of the isolates, and all of them were negative for *vanB* and $vanC_1$ genes amplification. The frequencies of *ermB* and *ermC* genes were 88.6% and 69.8%, respectively. The *ermA* and *msrA* genes were not detected in any of the strains.

Limitations

In this study, there were some limitations. First, small number of isolates was investigated. Second, *vanE* gene detection among *vanA*-negative isolates that were resistant to vancomycin was required.

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Conflict of Interest

The authors declare no conflict of interest.

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