# Antimicrobial Resistance and Virulence Determination of *Enterococcus* spp. Obtained from Hospital Environment in Iran

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**Research**

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# Abstract

**Background**: The role of the hospital environment as a source of pathogenic bacteria in recent studies has been poorly investigated. This study investigated the distribution of antimicrobial resistance genes and virulence determinants in *Enterococcus* species isolated from hospital environment in Sari, Iran.

**Method**: Overall, 90 enterococci strains were obtained from high touch surfaces of four hospitals in Sari, Iran. These environmental samples were obtained from bathroom, beds, tables, doorknobs, room keys, wheelchair and walls in the patient and staff's rooms. The resistance profile of the isolates was determined by disk diffusion method. Seven resistance genes and two virulence associated genes were evaluated molecularly by multiplex PCR.

**Results**: According to the PCR, 42 (46.66%) of them were *E. faecalis* and 48 (53.33%) others were detected as *E. faecium*. Also, 28 (66.6%) *E. faecalis* and 18 (37.5%) *E. faecium* isolates were multidrug-resistant (MDR). Among all 90 environmental isolates 54 (60%), 54 (60%), 8 (8.8%), 8 (8.8%), 60 (66.6%), 26 (28.8%), and 24 (26.6%) isolates contained *tetM*, *tetL, vanA, vanB, ermB, aac(6´)-Ie-aph(2´´)-Ia,* and *aph (3´)-IIIa*, respectively. Moreover, all isolates were investigated for the presence of virulence genes and 88 (97.7%) of isolates had *esp* gene, and 16 (17.7%) had *ace*.

**Conclusions**: This report showed that the environmental isolates of *Enterococcus* are the major sources of antibiotic resistance genes that can transfer them to the clinical isolates of bacteria in hospital settings. An effective following strategy should be organized to clearance and stop emergence of these pathogenic bacteria.


# Background

Enterococci are one of the most important opportunistic Gram-positive bacteria that have appeared as eminent pathogens causing a variety of clinical infections [1]. These organisms are human and animal intestinal normal-flora and they are even widely found in the environments [2]. Due to the capability to survive in unfavorable environmental conditions, enterococci have beheld a gradual enhancement in the emergence of nosocomial infections from different geographical regions, including many developing countries [3, 4]. Among all species of this genus, *Enterococcus faecium* shows an extensive range of antibiotic resistance, and *Enterococcus faecalis* is the most commonly carrying virulence factors and implicated in nosocomial infections [5]. These gram-positive bacteria can transfer the resistance genes to any type of bacteria. These gene-exchanges maybe happen in humans through the ingestion of bacterial contaminated foods, bacterial normal-flora in human reservoirs or the environmental bacteria [6, 7]. Due to inherently and acquired resistance to antimicrobials, treatment of the enterococcal infections is a major concern worldwide [8]. The acquisition of antibiotic resistance along with the ability of biofilm formation and the expression of various virulence factors by Enterococci have become them as the major nosocomial pathogens [9, 10]. Now, one of the most important phenotypes in the clinical and environmental isolates of enterococci is glycopeptide resistance which can be distinguished based on the level of resistance to vancomycin and teicoplanin, including VanA and VanB phenotypes [11]. Eight types of vancomycin resistance phenotypes are known in enterococci [12]. High-level aminoglycoside resistance phenotype caused by the production of aminoglycoside modifying enzymes in enterococci is generally interceded causing the elimination of the synergistic bactericidal effect of an anti-cell-wall-active agent and an aminoglycoside combination [13]. On the other hand, the emergence of a high level gentamicin resistance (HLGR) phenotype in enterococci is associated with the acquisition of the genes *aac(6´)-Ie-aph(2´´)-Ia* and *aph (3´)-IIIa*, causing resistance to all useful clinical aminoglycosides, which can be presented on the plasmids or chromosome of the bacteria [3]. Tetracycline resistance is one of the most common resistance phenotypes in enterococci which among all genes encoding this phenotype, the *tetL* and *tetM* presence is the most prevalent [14]. The *erythromycin ribosome methylation* (*erm*) genes encode methyltransferases that target special residues in 23S rRNA, from which the *ermB* gene, carrying by the conjugative transposon (Tn*917*), is the most prevalent ones in streptococci and enterococci [12, 15]. Along with resistance genes, virulence factors have been identified in enterococci involved in various stages of the infection [16]. The

enterococcus surface protein (ESP) is one of the most important virulence factors encoded by the *esp* gene in *E. faecalis* and *E. faecium* [17]. This protein mediates the biofilm formation by the enterococci that increases the bacterial survival in biopolymers and the emergence of the antimicrobial resistance [18]. One of the most important adhesion factors of enterococci is Ace protein (Adhesin to collagen of *E. faecalis*), found frequently in *E. faecalis* isolates [16]. Previous studies have focused on assessment of resistance genes and virulence factors of enterococci obtained clinical isolates. However, there is limited information about the role of the hospital environment as a source of pathogenic bacteria in our region. Our study aimed to assess the antimicrobial resistance and virulence genes in *Enterococcus* spp. isolated from hospital environment in Sari, north of Iran.

# Methods

### Sampling, Isolation, and Identification

The hospital environmental isolates of enterococci were collected during September 2018 to February 2018 from four main teaching and treatment hospitals (Buali Sina (a pediatric hospital), Imam Khomeini (an infectious disease center), Fatemeh Zahra (a heart diseases center), and Zare (a burn center)) in Sari, north of Iran. The isolates were collected from the bathroom, beds, tables, doorknobs, room keys, wheelchair and walls in the patient and staff's rooms. To sampling from the surfaces, we first rolled a cotton swab that was dipped in trypticase soy broth (TSB) (Merck, Germany) on the surfaces. The collected samples were cultured in TSB and incubated at 37˚C for 24 hours. Then, the bacteria grown in TSB were inoculated onto Slanetz and Bartley agar (m-Enterococcus agar) (Sigma, Germany) and incubated under aerobic conditions at 44 °C for 48 h. The isolates were determined as enterococci based on a series of standard microbiological tests [19]. To avoid the contamination in each step of our experiments, we selected randomly some swabs without rolling on the surfaces, and cultured them in TSB. The confirmation of the *Enterococcus* species was accomplished by polymerase chain reaction (PCR) method using the specific primers detecting D-alanine-D-alanine ligase encoding gene (*ddl* $_{E.\ faecium}$ *and ddl* $_{E.\ faecalis}$) (Additional file 1: Table S1). All isolates were stored in TSB broth with 15% glycerol at -70 ˚C until use.

### Antimicrobial susceptibility testing

The antimicrobial susceptibility testing was performed by disk agar diffusion method according to the clinical and laboratory standards institute [20] (CLSI) guidelines, against erythromycin (15 µg), vancomycin (30 µg), teicoplanin (30 µg), ampicillin (10 µg), linezolid (30 µg), tetracycline (30 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), nitrofurantoin (300 µg), chloramphenicol (30 µg), quinupristin-dalfopristin (15 µg**),** streptomycin (300 µg), and gentamicin (120 µg) (MAST, Merseyside, U.K). The streptomycin and gentamicin were used for detection of high-level aminoglycoside resistance (HLAR) in enterococci [20]. *E. faecalis* ATCC 29212 was used as a control strain in antimicrobial susceptibility testing.

### DNA extraction

Genomic DNAs of the enterococci environmental isolates were extracted by alkaline lysis method using the lysis buffer [sodium dodecyl sulphate (SDS) and NaOH] [21].

### Amplification of resistance genes and virulence factors

The multiplex-PCR were applied in order to distinguish for detection of *vanA*, *vanB, aac(6´)-Ie-aph(2´´)-Ia, aph(3´)-IIIa, tetL*, *tetM,* and *ermB* as the antibiotic resistance genes, and *esp* along with *ace* as the virulence genes of *E. faecalis* and *E. faecium* using the specific primers mentioned in Additional file 1: Table S1. These primers were designed in this study, and three sets of multiplex-PCR were done for detection of these genes. The first set was contained the *ermB, esp, vanA* and *vanB* genes, while the *aac(6´)-Ie-aph(2´´)-Ia,* and *aph(3´)-IIIa,* were set together in the second group and *tetL*, *tetM* and

*ace* genes were detected in the third set. The first set of the multiplex-PCR was performed in a final volume of 25 µl containing 600 ng of the template DNA, 5 pmol of each *ermB, vanA*, and *vanB* forward and reverse primers and 6 pmol of each *esp* primers, and 12.5 µl of master mix. The thermal profile was as follows, an initial denaturation step at 95 °C for 5 min followed by 30 cycles of denaturation at 95 °C for 30 s, annealing at 65 °C for 30 s, and an extension at 72 °C for 35s, followed by a final extension step at 72 °C for 10 min. Moreover, the second set of the multiplex-PCR was carried out in a final volume of 15 µl including 7.5 µl of master mix, 300 ng of the template DNA, 5 pmol of each *aac(6´)-Ie-aph(2´´)-Ia,* and *aph(3´)-IIIa* primers by a thermal condition including a 95 °C initial denaturation step for 5 min followed by 32 cycles of denaturation at 95 °C for 30 s, annealing at 65 °C for 30 s, and an extension at 72 °C for 35s, followed by a final extension step at 72 °C for 10 min. The third set of the multiplex-PCR was done in a final volume of 15 µl including 7.5 µl of the master mix, 300 ng of the template DNA, and 4 pmol of each *ace,* primers and 5 pmol of *tetL*, and *tetM* forward and revers primers. After an initial denaturation at 95 °C for 5 min, the amplification was performed in 32 cycles of denaturation at 95 °C for 30 s, annealing at 65 °C for 30 s, and extension at 72 °C for 1 min followed by a final extension at 72 °C for 10 min. Then, the PCR products were electrophoresed on 2% agarose gel containing safe stain.

### Statistical analysis

All data were statistically analyzed using chi-square and Fisher's exact tests while difference significance ($p < 0.05$) was determined using SPSS software (version 16).

## Results

### Identification of enterococcal isolates

From 388 hospital environmental samples, 90 (23.1%) isolates of enterococci were recognized to species level by common microbiological methods. Molecular analysis confirmed that 42 (46.66%) environmental isolates were *E. faecalis* and 48 (53.33%) others were detected as *E. faecium*. The sites of hospital environments which the bacteria were isolated are shown in Table 1. The most bacteria (26.7%) were obtained from the knob and the least isolation of bacteria (2.2%) were from the room keys.

Table 1
The distribution of the environment bacteria isolated from different sites of hospitals

| Source | Water Closet | Sweep | Faucet | Knob | Bed | Wall | Room keys | Table | Sinks | Wheelchair | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **N** | 12 | 12 | 8 | 24 | 6 | 10 | 2 | 4 | 8 | 4 | 90 |
| **(%)** | (13.3) | (13.3) | (8.8) | (26.6) | (6.6) | (11.1) | (2.2) | (4.4) | (8.8) | (4.4) | (100) |
| E. faecalis | 4 (33.3) | 10 (83.3) | 2 (25) | 12 (50) | 2 (33.3) | - | - | 4 (100) | 4 (50) | 4 (100) | 42 (46.6) |
| E. faecium | 8 (66.6) | 2 (16.6) | 6 (75) | 12 (50) | 4 (66.6) | 10 (100) | 2 (100) | - | 4 (50) | - | 48 (53.3) |

### Antimicrobial susceptibility

The antibiotic susceptibility profile of the isolates in this study is shown in Table 2. According to this data, the highest antibiotic resistance rate (62.2%) was shown against erythromycin, while 8.8% of the total isolates were resistant to teicoplanin. However, all isolates were susceptible to linezolid and nitrofurantoin. While all *E. faecalis* isolates were

susceptible to vancomycin and teicoplanin, but 10 (20.8%) and 8 (16.6%) isolates of *E. faecium* were detected as resistant to these glycopeptides, respectively. Surprisingly, 42 (46.6%) *Enterococcus* isolates were resistant towards quinupristin-dalfopristin. However, 46 (51.1%) environmental isolates of the present study were detected as multi-drug resistant (MDR). On the other hand, 28 (66.6%) *E. faecalis* and 18 (37.5%) *E. faecium* isolates were MDR.

Table 2
Antimicrobial susceptibility pattern of the tested isolates

| Antibiotics | No. (%) of isolates that were resistant | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | E. faecalis (n = 42) | | | E. faecium (n = 48) | | | Total (n = 90) | | |
| | R | I | S | R | I | S | R | I | S |
| Chloramphenicol | 14 (33.3) | | 28 (66.6) | 6 (12.5) | 4 (8.3) | 38 (79.1) | 20 (22.2) | 4 (4.4) | 66 (73.3) |
| Gentamycin | 10 (23.8) | | 32 (76.1) | 8 (16.6) | | 40 (83.3) | 18 (20) | | 72 (80) |
| Streptomycin | 18 (42.8) | | 24 (57.1) | 8 (16.6) | | 40 (83.3) | 26 (28.8) | | 64 (71.1) |
| Ampicillin | 6 (14.2) | | 36 (85.7) | 16 (33.3) | | 32 (66.6) | 22 (24.4) | | 68 (75.5) |
| Vancomycin | | | 42 (100) | 10 (20.8) | | 38 (79.1) | 10 (11.1) | | 80 (88.8) |
| Teicoplanin | | | 42 (100) | 8 (16.6) | | 40 (83.3) | 8 (8.8) | | 82 (91.1) |
| Erythromycin | 30 (71.4) | 6 (14.2) | 6 (14.2) | 26 (54.1) | 6 (12.5) | 16 (33.3) | 56 (62.2) | 12 (13.3) | 22 (24.4) |
| Tetracycline | 30 (71.4) | 2 (4.7) | 10 (23.8) | 22 (45.8) | | 26 (54.1) | 52 (57.7) | 2 (2.2) | 36 (40) |
| Ciprofloxacin | 14 (33.3) | 16 (38) | 12 (28.5) | 18 (37.5) | 14 (29.1) | 16 (33.3) | 32 (35.5) | 30 (33.3) | 28 (31.1) |
| Levofloxacin | 6 (14.2) | | 36 (85.7) | 16 (33.3) | | 32 (66.6) | 22 (24.4) | | 68 (75.5) |
| Quinupristin-dalfopristin | 30 (71.4) | 2 (4.7) | 10 (23.8) | 12 (25) | 12 (25) | 24 (50) | 42 (46.6) | 14 (15.5) | 34 (37.7) |
| Linezolid | | | 42 (100) | | | 48 (100) | | | 90 (100) |
| Nitrofurantoin | | | 42 (100) | | 2 (4.1) | 46 (95.8) | | 2 (2.2) | 88 (97.7) |

Abbreviations: R; resistant, I; intermediate resistant, S; susceptible.

## Prevalence of studied genes among *Enterococcus* strains

The prevalence of antibiotic resistance and putative virulence genes among the hospital environmental isolates of *Enterococcus* are shown in Table 3. The multiplex-PCR results showed that all gentamicin-resistant isolates were contained *aac(6´)-Ie-aph(2´´)-Ia* gene. Also, all HLGR isolates were carrying *aph (3´)-IIIa* gene, too. On the other hand, all isolates containing these aminoglycoside resistance genes were detected as streptomycin-resistant isolates, too. Also, all 52 (100%) tetracycline-resistant isolates and 2 (100%) intermediate resistant ones were carrying both *tetL* and *tetM* resistance genes, but any tetracycline susceptible isolates were contained these genes. Moreover, all 56 (100%) erythromycin-resistant isolates along with 4 (33.3%) intermediate resistant isolates were detected as *ermB* positive. Out of 10 isolates which were resistant to vancomycin, 8 (80%) isolates had both *vanA* and *vanB* genes, while theses 8 isolates were resistant against teicoplanin, too. The multiplex-PCR results on virulence factors of environmental enterococci showed that 88 (97.7%) and 16 (17.7%) isolates were contained the *esp* and *ace* virulence genes, respectively. Among them, 42 (100%) *E. faecalis* and 46 (95.8%) *E. faecium* isolates were carrying the *esp* gene, while 12 (28.5%) isolates of the *E. faecalis* and 4 (8.3%) isolates of the *E. faecium* were contained the *ace* gene. Totally, except *vanA* and *vanB* genes, other resistance and virulence genes investigated in this study were more prevalent in *E. faecalis* than *E. faecium*. On the other hand, there was a statistically significant correlation between the presence of antibiotic resistance genes and the rate of resistance to antibiotics ($P < 0.05$). The gene profiles of MDR and non- MDR enterococci investigated are shown in Table 4. According to this table, there was a significant correlation between the simultaneous presence of antibiotic resistance and virulence genes tested in this study and the emergence of MDR phenotype by the environmental isolates of enterococci. Surprisingly, all isolates which contained glycopeptide or aminoglycoside resistance genes were detected as MDR isolates, besides it seems that there was not a statistically significant relationship between the presence of biofilm encoding genes and the emergence of MDR phenotype.

Table 3
The prevalence of resistance and virulence genes among the tested isolates

| Genes | No. (%) of the isolates contained the genes | | |
|---|---|---|---|
| | E. faecalis (n = 42) | E. faecium (n = 48) | Total (n = 90) |
| tetM | 34 (80.9) | 20 (41.6) | 54 (60%) |
| tetL | 34 (80.9) | 20 (41.6) | 54 (60%) |
| vanA | - | 8 (16.6) | 8 (8.8%) |
| vanB | - | 8 (16.6) | 8 (8.8%) |
| ermB | 34 (80.9) | 26 (54.1) | 60 (66.6%) |
| aac(6´)-Ie-aph(2´´)-Ia | 18 (42.8) | 8 (16.6) | 26 (28.8%) |
| aph (3´)-IIIa | 14 (33.3) | 10 (20.8) | 24 (26.6) |
| esp | 42 (100) | 46 (95.8) | 88 (97.7%) |
| ace | 12 (28.5) | 4 (8.3) | 16 (17.7%) |

Table 4
The prevalence of different gene profile in MDR/non-MDR strains

| Gene profiles | No. (%) of *E. faecalis* (n = 42) | | No. (%) of *E. faecium* (n = 48) | | *P* value |
|---|---|---|---|---|---|
| | MDR isolates (n = 28) | Non-MDR isolates (n = 14) | MDR isolates (n = 18) | Non-MDR isolates (n = 30) | |
| tetM, tetL, aac(6´)-Ie-aph(2´´)-Ia, aph (3´)-IIIa, ermB, esp, ace **(n = 8)** | 6 (75) | - | 2 (25) | - | < 0.05 |
| tetM, tetL, aac(6´)-Ie-aph(2´´)-Ia, aph (3´)-IIIa, vanA, vanB, esp **(n = 4)** | - | - | 4 (100) | - | < 0.05 |
| tetM, tetL, aph (3´)-IIIa, vanA, vanB, esp, ace **(n = 2)** | - | - | 2 (100) | - | < 0.05 |
| tetM, tetL, aac(6´)-Ie-aph(2´´)-Ia, aph (3´)-IIIa, ermB, esp **(n = 10)** | 8 (80) | - | 2 (20) | - | < 0.05 |
| tetM, tetL, vanA, vanB, esp **(n = 2)** | - | - | 2 (100) | - | < 0.05 |
| tetM, tetL, aph (3´)-IIIa, ermB, esp **(n = 2)** | 2 (100) | - | - | - | < 0.05 |
| tetM, tetL, ermB, esp, ace **(n = 6)** | 4 (66. 6) | - | - | 2 (33.3) | NS |
| tetM, tetL, aac(6´)-Ie-aph(2´´)-Ia, aph (3´)-IIIa **(n = 2)** | 2 (100) | - | - | - | < 0.05 |
| tetM, tetL, ermB, esp **(n = 16)** | 8 (50) | 4 (25) | 2 (12. 5) | 2 (12. 5) | NS |
| tetM, tetL, esp, ace **(n = 2)** | - | 2 (100) | - | - | < 0.05 |
| esp, ace **(n = 14)** | - | 4 (28. 5) | - | 10 (71.4) | < 0.05 |
| esp **(n = 20)** | - | 4 (20) | - | 16 (80) | < 0.05 |
| **None of these genes (n = 2)** | - | - | 2 (100) | - | NS |

Abbreviations: NS; not statistically significant

# Discussion

Enterococci are the normal gastrointestinal flora of humans and animals that today, as a hospital pathogen, they can create a variety of diseases in hospitalized patients [22]. The spread of these potentially pathogenic enterococci from the hospital environment or other sources could enhance the outbreak of these strains in the human population as a risk factor to human health [23]. We found that, in concordant with another Iranian research on the clinical isolates of enterococci [8], all gentamicin-resistant isolates of our study were possessed the *aac(6´)-Ie-aph*(2´´)-*Ia* resistance gene. However, 20% of our hospital environmental isolates of enterococci were high-level gentamicin resistant (HLGR), and among all *Enterococcus* isolates of the present study, 26 (28.8%) isolates were contained this gene. This range was 42.8% in *E. faecalis* isolates, although 16.6% of *E. faecium* isolates were carrying this gene. However, a research in 2016, conducted in Iran [24], showed that 32.8% and 67.2% of their *E. faecium* and *E. faecalis* clinical isolates were contained *aac(6´)-Ie-aph(2´´)-Ia*, respectively, while the prevalence of *aph(3´)-IIIa* gene was 77.3% and 22.7%, respectively, which both of them were more prevalent than our study. However, another Iranian study on burn patients showed that 65.2% of their *E. faecalis* isolates were detected as HLGR, while 47.8% of them had *aac(6´)-Ie-aph(2´´)-Ia* gene [25]. This data confirmed that the prevalence of these genes in clinical *Enterococcus* isolates is higher than the environmental isolates, may be due to the high prescription of aminoglycosides for treatment of infections caused by Gram-negative bacteria and enterococci in Iran. This was in concordant with a study carried out in India [9], from which *aac(6´)-Ie-aph(2´´)-Ia* and *aph* (3´)-*IIIa* genes were detected in 39.5% and 37.5% of their clinical isolates, respectively. Also, the prevalence of *aac(6´)-Ie-aph*(2´´)-*Ia* in enterococci isolated from non-hospital samples and surface waters in Thailand were reported as 0.9% and 1.6%, respectively [22]. While, the prevalence of *E. faecalis* and *E. faecium* isolated from wastewater containing this gene in Tunisia were 5.8% and 3.7%, respectively [26]. The emergence of high-level gentamicin resistance in enterococci, and concurrent resistance to ampicillin and vancomycin due to the role of the same plasmid in the transfer of their genes were reported in some studies [3, 27]. Considering such a finding, the detection of HLGR strains together with vancomycin-resistant enterococci in this research displays an alarming situation in our region. Vancomycin-resistant enterococci (VRE) have led to hospital prevalence worldwide, and *vanA* gene has connected to methicillin-resistant *S. aureus* [28]. While we detected this gene just in 8.8% of *E. faecium* isolates and our *E. faecalis* ones were negative, in an Australian research in 2012 [23], any vancomycin-resistant strains isolated from water were contained this gene. However, some of their *E. faecium* and *E. faecalis* clinical isolates were carrying *vanA* and *vanB* genes, indicating the more prevalence in clinical isolates than environmental ones. Moreover, a research conducted in Iran [25], exhibited that all of their *E. faecalis* isolated from burned patients were susceptible to vancomycin and the *vanA* and *vanB* genes were not found. This range in Italy was 10.7% and 0.7% among meat and environmental isolates of glycopeptide resistant *Enterococcus* (GRE), respectively [29]. Also, we detected that all of our isolates carrying *vanA* and *vanB* genes were detected as *tetL*, *tetM*, and *esp* positive, while 4 of them were contained *aac(6´)-Ie-aph(2´´)-Ia* and *aph (3´)-IIIa* genes, too, and 2 isolates had all resistance and putative virulence genes tested in the current study. The most common antibiotic resistance phenotype in enterococci is tetracycline resistance [30]. In the present study, 60% of enterococci isolates were positive for *tetM* and *tetL*, this range in animal meat samples and clinical specimens were different in research conducted by Ebru Sneb Yılmaz et al. [30]. In addition, in Tunisia, just 3.7% and 11.7% of *E. faecium* and *E. faecalis* isolated from wastewater and surface water samples carrying the *tetM* gene [26]. In the most studies on enterococci in the world, similar to the present research, the resistance rate to erythromycin is equal to the level of resistance to tetracyclines, and among them, the *ermB* resistance gene was the most prevalent in both *E. faecalis* and *E. faecium* erythromycin-resistant isolates [2, 14, 30, 31]. In the current study, in addition to all 56 erythromycin-resistant environmental isolates of enterococci, 4 intermediate resistant isolates were contained the *ermB* gene, too. This *ermB* high prevalence was concordant to all above-mentioned studies worldwide, may be due to the role of mosaic plasmids harboring Tn*1546-ermB* element transferable among *S. aureus* and *Enterococcus Spp.*, as a developing problem requiring constant monitoring [32]. Meanwhile, almost 43% of the enterococci isolated from the waste and surface waters were carrying this gene [26], but the prevalence of *ermB* gene among the erythromycin-resistant clinical isolates of enterococci in Spain [2] was almost similar to our study [33]. However, the prevalence of *ermB* gene in *E. faecalis* isolated from burned patients was 54.3% [25], in concordant to Bulgarian research [34], which 59.5% of their clinical isolates contained this gene, while

just 4.3% of their *E. faecium* isolates were *ermB* positive. According to the prescription of erythromycin in different countries, the prevalence of *ermB* gene in enterococci is varied, as just 33% of the clinical *E. faecalis* isolated from Mexico were carrying the *ermB* gene, while all of them were susceptible against vancomycin and 62% of their isolates were resistant to tetracycline [35]. On the other hand, in a study conducted in Turkey, the higher prevalence of tetracycline resistance genes in enterococci isolated from chicken meats than beef was observed about the *ermB* gene, too [30]. However, 76% and 83.8% of *E. faecalis* and *E. faecium* isolated from slaughter pigs in Australia were detected as the *ermB* positive, respectively, in 2011 [14]. These data indicate that the hospital environments, waters and animal isolates of enterococci could be the major sources of the *ermB* resistance gene transferring it to the clinical isolates by plasmids. However, among hospital environmental isolates in Tunisia, 97.3% of them were resistant to erythromycin and all tetracycline and vancomycin-resistant, were contained *tetM* and *vanA* genes, respectively [31]. Considering the antibiotic resistance pattern of the enterococcal isolates, we found that linezolid and nitrofurantoin are the most effective antibiotics for treatment of possible infections caused by our environmental isolates in immunocompromised hospitalized patients. However, even erythromycin or tetracycline intermediate resistant and non-HLAR isolates of the present study were carrying the resistance genes. Another thing to consider in this study is that we also tested ampicillin in this study, although the bacterium is almost inherently resistant to this antibiotic. We had two reasons for this: 1) because the CLSI has recommended this drug for antibiotic susceptibility testing. 2) because in our area the use of this antibiotic has reached the lowest possible level, and we wanted to investigate whether the rate of enterococci ampicillin resistance could be reduced. We concluded that this is possible, because only 24.4% of our isolates were resistant to this antibiotic. The *esp* gene was defined as characteristic to hospital strains and claimed to be greatly correlated to the capability of the isolates to cause health-care associated infection [36]. The importance of the presence of *esp* and *ace* genes in the biofilm synthesis process was highlighted by the results of a study conducted by Papadimitriou, et al., 2015 [37]. In the present study, 97.7% of the isolates had *esp* gene, while 17.7% of them were contained the *ace* gene. According to the study conducted in China, *ace* gene was found in 92% of environmental enterococcal strains (23/25). This difference may be due to the various samples which used in two types of research [38]. However, in a study conducted in Mexico, 39% of the *E. faecalis* clinical isolates were *ace* positive [35]. *E. faecalis* and *E. faecium* can survive outside the host for a long time using the biofilm construction, as well as being able to resist routine cleaning and antibiotics due to the possession of resistance genetic elements, contaminate the environments and transfer to hospitalized patients by contact with the medical equipments, other patients, and contaminated surfaces [31, 39].

# Conclusions

According to the present study, antibiotic resistance has a relatively high prevalence in the environmental isolates of *E. faecalis* and *E. faecium*. Beside, a large source of antibiotic resistance and biofilm encoding genes was identified from enterococci isolated in this study. These genetic elements can be transmitted to other bacteria resulting in the increase of antibiotic resistance level in a healthy human community and consequently in the hospital environmental bacteria. As a result, the treatment of infections caused by these resistant microorganisms is becoming more and more difficult.

# Abbreviations

MDR: multidrug-resistant, PCR: polymerase chain reaction, erm: erythromycin ribosome methylation, Ace: Adhesin to Collagen of E. Faecalis, HLGR: high level gentamicin resistance, TSB: trypticase soy broth, ESP: enterococcus surface protein, ddl: D-alanine-D-alanine ligase, CLSI; clinical and laboratory standards institute, SDS: Sodium Dodecyl Sulphat, VRE: Vancomycin-resistant enterococci,

# Declarations

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### Authors' contributions

HG: Design of the study and supervision. SA: collected the data, cultured the samples and performed experiments. RV Advisor in the study and contributed to the analysis of the data in collaboration with MG. MN: Assisted in molecular examinations and edited the manuscript. MG drafting of the manuscript in collaboration with HG. All authors read and approved the final manuscript.

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### Availability of data and materials

All data generated or analyzed during this work are included in this published article.

### Ethics approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Competing interests

The authors declare that they have no competing interests.

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