



Antimicrobial Resistance, Virulence Factors, Diversity, and Genetic Clonality of *Enterococcus* Species from Clinical Samples

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Abstract

Over the past decade, the emergence of antibiotic-resistant enterococci has posed critical challenges to the treatment of nosocomial infections around the world, and VRE infections have been associated with mortality and morbidity worldwide, especially in immunocompromised individuals. A total of 285 *Enterococcus* isolates were collected from patients referring to three major hospitals in Tehran from July to December 2012, and species were identified using biochemical tests and PCR. PCR assay and antibiotic susceptibility testing were performed to detect *Enterococcus* species, virulence factors, and resistance genes as well as to evaluate drug resistant isolates, respectively. The clonality of the isolates was also determined by PFGE. The isolates were found to consist of *E. faecalis* (65%) and *E. faecium* (31%) species. According to the results, 39 and 53% of *E. faecalis* and *E. faecium* isolates were resistant to the most commonly used antimicrobial agents, respectively. Also, *acm* (23, 64%) and *hyl* (6, 86%) were the most prevalent genes in *E. faecalis* isolates, while the most prevalent virulence genes in *E. faecium* isolates were *cyt* (7, 100%), *gel* (30, 97%), and *asa* (29, 97%). PFGE revealed a high heterogeneity among 50 VRE isolates. The emergence of multidrug-resistant and heterogeneous populations of enterococci is considered as a serious threat of global concern.

Keywords: *Enterococcus*; Resistance; Antimicrobials; PFGE

Abbreviations

BSI: Bloodstream Infections; NNIS: National Nosocomial Infection Surveillance; GRE: Glycopeptide-resistant Enterococci; VRE: Vancomycin-resistant Enterococci; ICU: Intensive Care Unit; PYR: Pyrrolidonyl Arylamidase; MDR: Multidrug Resistant; BHI: Brain Heart Infusion; PFGE: Pulsed-field Gel Electrophoresis

Introduction

The increasing emergence of enterococci as one of the chief causes of nosocomial infections and their high capacity to receive and transfer antibiotic resistance genes have brought members

of this genus, especially *Enterococcus faecium*, to the forefront of hospital infection control issues. Enterococci are considered as the second leading cause of urinary tract infections [1] as well as one of the causes of nosocomial UTI (10%), intra-abdominal and pelvic abscesses, and post-surgery wound infections [2,3]. Studies have shown that *Enterococcus* is the fourth and fifth most common cause of bloodstream infections (BSI) in North America (10.2%) and Europe (7.2%), respectively, as assayed by the SENTRY Antimicrobial Surveillance scheme [4]. According to the National Nosocomial Infection Surveillance (NNIS), the incidence of glycopeptide-resistant enterococci (GRE) increased from 0.3% in 1989 to 25.9% in 1999 in the USA, which could be considered as a serious threat [5].

In recent years, the emergence of vancomycin-resistant enterococci (VRE) has posed critical challenges for practitioners responsible for controlling hospital infections as well as for clinicians remedying patients with enterococcal nosocomial infections [6], and VRE infections have been associated with mortality and morbidity worldwide, especially in immunocompromised individuals; also, nearly 25% of enterococcal infections in intensive care unit (ICU) patients have been detected to be VRE infections [7]. Increased consumption of vancomycin for the treatment of methicillin-resistant staphylococcal infections has been identified as a prominent factor in the development of VRE, among other factors such as clonal dissemination [8]. Enterococcal infections in hospitals are mostly clonal with epidemic-virulent clonal complex 17 (CC-17) lineage of *E. faecium* [9]. Some of which are caused by single strain outbreak, while others are caused by simultaneous clonal spread of endemic clusters existing in hospitals [8]. Control and prevention programs designed for hospital-acquired infections caused by enterococci concentrate on procedures that hinder cross-transmission between cases and control antimicrobials consumption [10].

According to diverse reports on VRE prevalence rate in health centers in Iran, diseases and mortality rate due to enterococcal infections in Iran are increasing every day. One of the most important reasons is that the proper antimicrobial treatment of enterococcal infections has gradually become more difficult for Iranian physicians due to the lack of sufficient information about the prevalence of VRE and increased antibiotic resistance in these strains [11]. Therefore, this survey aimed to investigate the frequency of *Enterococcus* species, pathogenic factors, and VRE isolates as well as VRE antibiotic resistance pattern in three different healthcare departments in Tehran.

Material and Methods

Samples collection and identification

In this cross-sectional study, 285 *Enterococcus* isolates were collected from patients referring to three major hospitals in Tehran from July to December 2012. Samples (taken from urine, catheter, BAL, blood and wound) were instantly cultured on m-*Enterococcus* agar, bile esculin agar, and blood agar media at 37°C. *Enterococcus* genus identification was performed based on the following microbiological tests: Gram stain, catalase, presence of pyrrolidonyl arylamidase (PYR), and growth on bile-aesculin agar medium with 6.5% NaCl. Other tests performed in this study were as follows:

motility, arginine decarboxylation in Moeller decarboxylase media, pyruvate utilization, and carbohydrates fermentation tests (Arabinose, Raffinose, Mannitol, Ribose).

Antibiotic susceptibility testing

All *Enterococcus* isolates were tested for susceptibility to Vancomycin 30 (µg), Gentamicin 120 (µg), Erythromycin 15 (µg), Amoxicillin 25 (µg), Ciprofloxacin 5 (µg), Streptomycin 30 (µg), Linezolid 30 (µg), Synercid 15 (µg), Ampicillin 10 (µg), Chloramphenicol 30 (µg), Nitrofurantoin 300 (µg) and Rifampin 5 (µg) by the Kirby-Bauer disk diffusion method (MAST, United Kingdom) [12]. Resistance breakpoints to a particular antibiotic were determined using standard reference values. Isolates that were resistant to at least one antibiotic in three or more antibiotic classes were classified as multidrug resistant (MDR) [13]. *Staphylococcus aureus* ATCC 25923 was included as a quality control organism for antimicrobial susceptibility determination.

DNA extraction

Enterococcus strains were grown overnight at 35°C on Brain Heart Infusion (BHI) agar. Then two or three colonies were scraped from the surface of each agar culture plate, and the pellet was then used for DNA extraction by High Pure PCR Template Preparation Kit (Roche Diagnostic GmbH, Mannheim, Germany) [14].

PCR assay for the detection of *E. faecium*, *E. faecalis*, and virulence factors

According to table 1, all of the 285 enterococci isolates were analyzed for the presence of specific genes by PCR. Singleplex PCR was carried out for the amplification of the D-Ala-D-Ala ligase (*ddl*) gene to detect *E. faecium* and *E. faecalis*, Singleplex PCR was also carried out for the amplification of *vanA* and *vanB1* as resistance genes, virulence factors were detected using multiplex PCR for *acm*, *gelE*, *Hyl*, *asa*, *esp*, and *cyt* genes. PCR reactions were performed in a 25 µL volume consisting of 2.5 µL of PCR Buffer (10X), 0.6 µL of MgCl₂ (50mM), 0.4 µL of dNTP Mix (10 mM), 0.4 µL of primer 1 (100 pm/µL), 0.1 µL of *Taq* DNA polymerase (5U/µL), 1 µL of Template DNA, and 19.6 µL of ddH₂O.

PCR cycling conditions were as follows: an initial denaturation step of DNA at 95°C for 4 min, followed by 30 cycles of denaturation at 95°C for 30s, primer annealing at 50 to 63°C (according to table 1) for 1 min, DNA synthesis step at 72°C for 1 min, and final DNA

Virulence Genes	Primer Sequence (5'-3')	Size Products (bp)	Annealing Temperature	Reference
<i>ddl(E. faecalis)</i>	F-TTGAGGCAGACCAGATTGACG R- TATGACAGCGACTCCGATTCC	658	50 °C	[30]
<i>ddl(E. faecalis)</i>	F- ATCAAGTACAGTTAGTCT R- ACGATTCAAAGCTAACTG	941	50 °C	[31]
<i>acm</i>	F- GGCCAGAAAACGTAACCGATA R- AACCCAGAAGCTGGCTTTGTC	135	52 °C	[32]
<i>gelE</i>	F- TATGACAATGCTTTTTGGGAT R- AGATGCACCCGAAATAATATA	213	52 °C	[32]
<i>Hyl</i>	F- ACAGAAGAGCTGCAGGAAATG R- GACTGACGTCCAAGTTCCAA	276	52 °C	[33]
<i>asa</i>	F- GCACGCTATTACGAACTATGA R- TAAGAAAGAACATCACACGA	375	52 °C	[33]
<i>Esp</i>	F- AGATTTTCATCTTTGATTCTTGG R- AATTGATTCTTTAGCATCTGG	510	52 °C	[34]
<i>Cyt</i>	F- AGATTTTCATCTTTGATTCTTGG R- AATTGATTCTTTAGCATCTGG	688	52 °C	[34]
<i>VanA</i>	FCATGAATAGAATAAAAGTTGCAATA R- CCCCTTTAACGCTAATACGATCAA	1030	54 °C	[34]
<i>VanB</i>	FGTGACAAACCGAGGCGAGGA R-CCGCCATCCTCTGCAAAAAA	433	54 °C	[34]

Table 1: Primers used to detect *E. faecium* and *E. faecalis*, resistance genes and virulence factors.

synthesis at 72°C for 10 min [15]. Eventually, 10 µL of PCR products were analyzed by electrophoresis (120 mV for 80 min) on a 1% agarose gel using safe stain. The agarose gel was illuminated by UV, and DNA bands were finally analyzed and recorded.

Pulsed-field gel electrophoresis (PFGE)

Enterococcus isolates were analyzed by pulsed-field gel electrophoresis (PFGE). First all selected *Enterococcus* strains grew in BHI broth overnight with shaking at 37°C. All the cultures were

centrifuged and cells were re-suspended in 5 ml of TE buffer (10 mM Tris, 0.1 mM EDTA [pH 8.0]), blended with an equalized volume of 2% low melting agarose (Bio-Rad, Hercules, CA) in distilled water and poured down into a plug mold. Then, all the plugs were treated in lysis buffer including lysosome (1 mg/ml) and RNase (5 mg/ml), (6 mM Tris [pH 7.5], 100 mM EDTA [pH 7.5], 1M Na-Cl, 0.5% Brij-58, 0.2% sodium deoxycholate, and 1% sodium lauryl sarcosine) overnight at 37°C. Mixtures were supplanted by ES solution (0.5 M EDTA [pH 9.5], 1% sarcosine) and then ESP solution

(0.5 M EDTA [pH 9.5], 1% sarcosin, and 0.5 mg of proteinase K/ml) and were incubated 48h at 50°C. Then the plugs were washed with TE buffer and stored at 4°C. Afterwards, the plugs were digested with 20 U SmaI (Roche, Manhiem, Germany) and settled in the wells of 1% agarose in 0.5% × TBE and electrophoreses with switch times ramped from 5 to 35 s at 6 V with a run time of 27h at 14°C in the Bio-Rad CHEF-DRIII system. DNA from *Salmonella choleraeraesuis* serotype Branderup H9812 (Pulsenet, www.cdc.gov/pulsenet) were used as molecular size marker. The gels were stained with ethidium bromide and visualized the restricted DNA with ultraviolet light PFGE patterns then analyzed and interpreted using GelCompar II software Version 6.5 (Applied Maths, Belgium). The genetic similarities between the fingerprints were determined using Dice coefficients and unweighted pair-group method with arithmetic mean (UPGMA) [16].

Statistical analyses

Data were statistically analyzed by employing Pearson chi-square test for dependent samples or Fisher’s exact test where appropriate using SPSS software Version 19.0. A p-value of < .05 was considered as statistically significant.

Results and Discussion

Out of 285 *Enterococcus* isolates obtained from 5560 hospitalized patients with different age groups (from infants to 93 years), 168 (65%) were from Milad hospital, 67 (24%) were from Shariati hospital, and 32 (11%) were from Tehran Heart Center. The majority of the isolate were recovered from urine (196, 69%) and blood (35, 12%) samples of both males (48%) and females (52%) (Table 2).

	Urine	Blood	Wound swab	Catheter	Endotracheal tube	BAL	Abscess	Ascitic fluid	Pleural fluid	Others
<i>E. faecalis</i>	130	18	15	4	1	5	2	1	1	4
<i>E. faecium</i>	53	15	4	6	2		1	1		2
Others	13	2	3		1			1		1
Total	196	35	22	10	4	5	3	3	1	7

Table 2: Distribution of *Enterococcus* strains in different clinical samples.

In this study, two different *Enterococcus* species were identified based on the standard guideline of species identification. Most of the isolates were *E. faecalis* (65%), followed by *E. faecium* (31%) and other strains (4%). The distribution of *Enterococcus* strains in different clinical wards are shown in table 3.

Antibiotic susceptibility testing

Resistance of *E. faecium* isolates to antimicrobial agents tested in this study based on DDT (disk diffusion technique) was higher than that of *E. faecalis* isolates, except for ampicillin and amoxicillin. The highest susceptibility of *E. faecium* isolates was observed to ampicillin (79%) and amoxicillin (67%), while the highest susceptibility of *E. faecalis* isolates was observed to nitrofurantoin (86%) and synergid (86%). Antimicrobial resistance patterns of *Enterococcus* isolates are shown in table 4.

Out of 285 *Enterococcus* isolates, 50 (17.5%) were found to be VRE using DDT. Overall, the prevalence of vancomycin-resistant *E.*

faecium (37, 74%) isolates were higher than that of vancomycin-resistant *E. faecalis* (13, 26%) isolates. In this study, 38 (13.3%) isolates were multidrug resistant (MDR). The prevalence of MDR isolates was higher among *E. faecium* (20, 53%) isolates compared to *E. faecalis* (15, 39%) isolates.

Prevalence of virulence genes among *Enterococcus* strains

Figure 1 shows the prevalence of six virulence genes identified in the sample. According to the results, *acm* (23,64%) and *hyl* (6, 86%) were the most prevalent genes in *E. faecalis* isolates, while the most prevalent virulence genes in *E. faecium* isolates were *cyt* (7, 100%), *gel* (30, 97%), and *asa* (29, 97%). Only two (6%) *E. faecalis* isolates carried *asa* and *gel* genes, and *cyt* was not detected in *E. faecalis* isolates.

Pulsed-field gel electrophoresis (PFGE)

The clonality of 50 VRE *E. faecium* and *E. faecalis* isolates were analyzed by PFGE. Then found among all VRE isolates 8 types con-

	<i>E. faecalis</i>	<i>E. faecium</i>	Others	Total
CCU	16	4	4	24
ICU	24	26	2	52
NICU	1	0	0	1
Surgery	9	6	2	17
Laboratory	2	0	0	2
Children	3	9	1	13
Hospitalization	14	5	0	19
Bone marrow Transplant	4	3	0	7
Internal	14	5	1	20
Outpatient	47	16	5	68
Glands	2	0	0	2
Heart	3	1	0	4
Kidney	19	4	3	26
Digestion	4	4	0	8
Neurology	0	2	0	2
Emergency	18	0	2	20
Total	180	85	20	285

Table 3: Distribution of *Enterococcus* strains in different clinical wards.

belonging to 2 single types (ST, ST2). Figure 2 shows the representative PFGE patterns. Three dominant clusters (CT, CT6, CT8) containing 7, 8 and 8 isolates respectively. In CT1 and CT2 types, there were some isolates from all three hospitals, suggesting the rotation of this bacterium in these three hospitals. The isolates typing results in this study showed that there was the possibility of in-hospital transfer of strains.

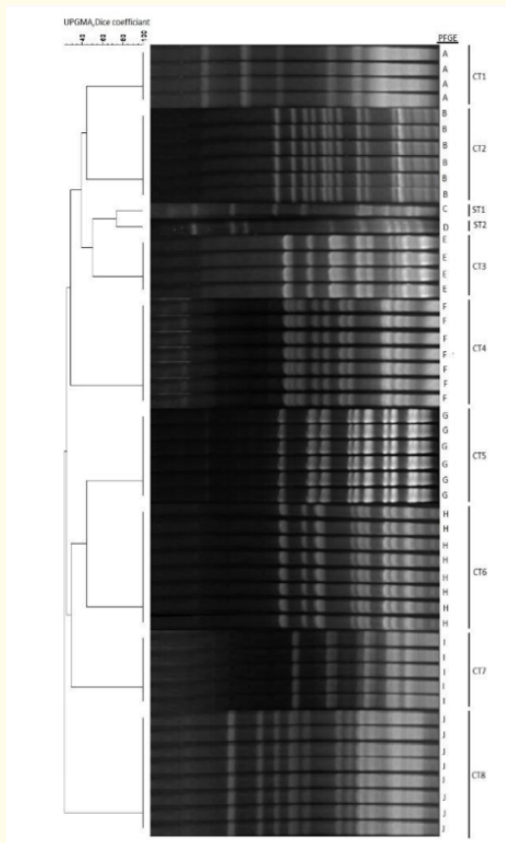


Figure 2: Dendrogram based on pulsed-field gel electrophoresis (PFGE) Small restriction pattern analysis of VRE isolates.

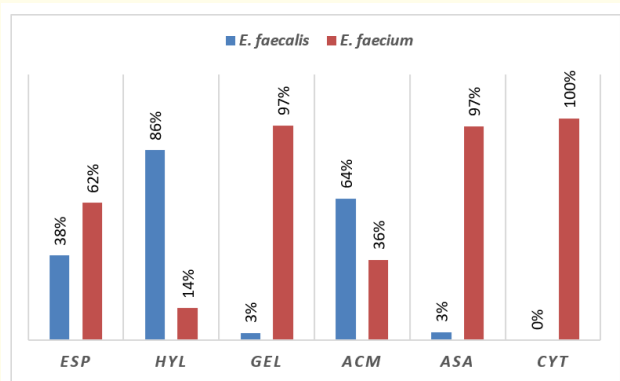


Figure 1: Prevalence and distribution of virulence factors in *Enterococcus* isolates.

taining 50 identical isolates with more than 80% similarity (Figure 2). The remaining two types contained highly diverse isolates

Enterococcus are part of the normal flora of the oral cavity, intestinal tract, vagina, etc. They are associated with different types of infections, especially as nosocomial pathogens. The emergence of enterococcal resistance and tolerance against antimicrobial agents is a serious concern worldwide [17]. Therefore, it is important to know *enterococcus* resistance patterns and antibiogram profile. In

this study, 285 *Enterococcus* isolates were recovered from 5560 clinical specimens during a six-month study period. The samples examined were urine, blood, catheter, BAL, and wound samples taken from hospitalized patients. The highest number of isolates was obtained from urine (69%), followed by blood (12%) samples. Enterococcal bacteraemia mortality rates have been reported from 19 to 48%. It is generally accepted that enterococcal bacteraemia often occurs in patients with blood stream and surgical site infections. *Enterococcus* are next to *Staphylococcus aureus* and coagulase-negative *Staphylococcus* causing clinically significant infections in the bloodstream and other tissues; therefore, their isolation from bloodstream is very important and is often very difficult for a laboratory expert [18]. Furthermore, out of 285 isolates, 52% were non hemolytic, and 48% were β hemolytic. Hemolytic activity plays an important role in the pathogenesis of enterococci. The majority of the β hemolytic isolates were obtained from urine specimens. Most of the isolates investigated were *E. faecalis* (65%), followed by *E. faecium* (31%) and other strains (4%). Like other studies in Iran, *E. faecalis* was more prevalent among the isolates investigated in this study [19]. In a study in India, out of 80 *Enterococcus* strains, 69 were *E. faecalis*, 10 were *E. faecium*, and one was *E. durans* [20]. When enterococci are isolated in different geographical regions, there might be variation in species distribution. *E. faecalis* and *E. faecium* are the most frequent enterococcal species isolated from different clinical specimens, they are responsible for 90% of nosocomial infections [21]. The prevalence of resistance to chloramphenicol, nitrofurantoin, and synergid in *E. faecalis* isolates as well as to ampicillin and amoxicillin in *E. faecium* isolates was low, indicating that these antimicrobial agents could be considered as therapeutic options. Resistance rate to ampicillin among *E. faecalis* isolates was higher than in *E. faecium* isolates (74% versus 21%); this finding is different from the finding of another study by Saifi, et al. [19]. *Enterococcus* could overexpress low-affinity penicillin-binding proteins against β -lactams. In addition, plasmid mediated β -lactamases have been shown to be responsible for resistance in these strains, allowing *Enterococcus* to be intrinsically resistant to penicillin [22]. Dalfopristin resistance in *E. faecalis* strains is conferred through an efflux pump that seems to be intrinsic in this species. MDR strains in the present study were reported to be 13.3%. The prevalence of MDR isolates was higher in *E. faecium* (20, 53%) than in *E. faecalis* (15, 39%) isolates. Numerous factors such as antimicrobials overuse, genetic mutations, and disease control measures contribute to the development of multidrug resistance

phenotypes [23]. MDR *enterococcus* exhibiting high resistance to penicillin, glycopeptides, fluoroquinolones, and aminoglycosides have been identified as major causes of nosocomial infections. Isolation of several MDR enterococci strains have a great concern due to the limitations in clinical use of antimicrobials, especially the use of their synergistic combinations which are often needed for the treatment of enterococcal infections [24].

There are some reports about increased resistance of *enterococci* to ciprofloxacin [25]. High resistance was observed to ciprofloxacin and nitrofurantoin in the present study. It may be due to the widespread use of these antibiotics for UTI as the first line treatment. Besides, the prevalence of vancomycin resistance in *E. faecium* (65%) isolates was higher than in *E. faecalis* (26%) isolates, which was in accordance with teicoplanin resistance pattern in these isolates. Moreover, both species of *E. faecium* and *E. faecalis* showed resistance to linezolid. Most of the *E. faecium* isolates were resistant to quinupristin-dalfopristin (83%) and linezolid (60%).

Besides, antibiotic-resistant *Enterococcus* spp. consisted potential virulence factors. It is worth noting that several of these enterococcal virulence traits are genetically transmissible through some genes located on specific regions of the genome, specifically in an area called the "pathogenicity islands" [17]. For example, exotoxin cytolysin (*cyt*) exhibits bacteriocidal characteristics towards Gram-negative bacteria and toxic properties towards erythrocytes, leukocytes, and macrophages. Aggregation substance (*asa1*) as an enterococcal surface protein participates in the formation of biofilm, which plays a significant role in the genetic material exchange between cells and improves their antibiotics resistance. In *E. faecium* strains, the presence of the *esp* gene is correlated with resistance to ampicillin, ciprofloxacin, and imipenem. Hyaluronidase (*hyl*) plays an important role in destroying mucopolysaccharides in cartilage and connective tissues, and subsequently, in spreading bacteria [26]. Collagen-binding adhesin and gelatinase (*gelE*) also play an important role in increasing pathogenicity [27]. However, *acm* (23, 64%) and *hyl* (6, 86%) were the most prevalent genes in *E. faecalis*, while the most prevalent virulence genes in *E. faecium* were *cyt* (7, 100%), *gel* (30, 97%) and *asa* (29, 97%). Only two (6%) *E. faecalis* isolates carried *asa* and *gel* genes, and *cyt* was not detected in *E. faecalis* isolates. Other studies have also reported that the *cyt* and *gel* genes were more often carried by *E. faecalis* strains. But in the present study, *cyt*, *asa*, and *gel* were more common in *E. faecium*.

The presence of multiple microorganisms at the site of infection usually causes biofilm-associated infections [28].

PFGE profiles indicated that high heterogeneity amongst *E. faecalis* and *E. faecium* clinical strains isolated from different patients in different wards, might be an indication of the possibility of cross-transmission of strains between patients in different wards within the hospitals. Similar studies findings exhibited a high genetic diversity amongst these isolates. Because of a high degree of differentiation between isolates, PFGE is considered as the “gold standard” for assessing hospital outbreaks[29]. Two common types, CT1 and CT2 contained some isolates from all three hospitals, suggesting the rotation of this bacterium in these three hospitals. The isolates typing results in this study showed that there was the possibility of inter-hospital transfer of strains.

Conclusion

In conclusion, in addition to identification of these strains, preventing and controlling the spread of multidrug-resistant enterococcal infections in hospitals require a coordinated effort between various departments, and this could only be achieved by monitoring the use of antibiotics, educating hospital staff, early detection and reporting by laboratories, and strict implementation of appropriate infection control measures.

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Conflict of Interest

The authors have declared that no competing interests exist.

Bibliography

1. Kumpu Minna, *et al.* “Effect of live and inactivated *Lactobacillus rhamnosus* GG on experimentally induced rhinovirus colds: randomised, double blind, placebo-controlled pilot trial”. *Beneficial Microbes* 6.5 (2015): 631-639.
2. Mascini EM and M J M Bonten. “Vancomycin-resistant enterococci: consequences for therapy and infection control”. *Clinical Microbiology and Infection* 11 (2005): 43-56.
3. Schouten M A., *et al.* “Prevalence of vancomycin-resistant enterococci in Europe”. *European Journal of Clinical Microbiology and Infectious Diseases* 19.11 (2000): 816-822.
4. Biedenbach Douglas J., *et al.* “Occurrence and antimicrobial resistance pattern comparisons among bloodstream infection isolates from the SENTRY Antimicrobial Surveillance Program (1997–2002)”. *Diagnostic Microbiology and Infectious Disease* 50.1 (2004): 59-69.
5. Nelson R R S., *et al.* “Isolation and characterization of glycopeptide-resistant enterococci from hospitalized patients over a 30-month period”. *Journal of Clinical Microbiology* 38.6 (2000): 2112-2116.
6. Feizabadi Mohammad Mehdi., *et al.* “Drug resistant patterns of enterococci recovered from patients in Tehran during 2000-2003”. *International Journal of Antimicrobial Agents* 24.5 (2004): 521-522.
7. Christidou A., *et al.* “Emergence of vancomycin-resistant enterococci in a tertiary hospital in Crete, Greece: a cluster of cases and prevalence study on intestinal colonisation”. *Clinical Microbiology and Infection* 10.11 (2004): 999-1005.
8. Chavers L S., *et al.* “Vancomycin-resistant enterococci: 15 years and counting”. *Journal of Hospital Infection* 53.3 (2003): 159-171.
9. Klare I., *et al.* “Spread of ampicillin/vancomycin-resistant *Enterococcus faecium* of the epidemic-virulent clonal complex-17 carrying the genes *esp* and *hyl* in German hospitals”. *European Journal of Clinical Microbiology and Infectious Diseases* 24.12 (2005): 815-825.
10. Fridkin Scott K., *et al.* “The effect of vancomycin and third-generation cephalosporins on prevalence of vancomycin-resistant enterococci in 126 US adult intensive care units”. *Annals of internal medicine* 135.3 (2001): 175-183.
11. Talebi Malihe., *et al.* “*Enterococcus faecium* from meat, chicken and cheese”. *Jundishapur Journal of Microbiology* 8.4 (2015).
12. Humphries Romney M., *et al.* “CLSI methods development and standardization working group best practices for evaluation of antimicrobial susceptibility tests”. *Journal of Clinical Microbiology* 56.4 (2018).
13. Diekema Daniel J., *et al.* “Antimicrobial resistance trends and outbreak frequency in United States hospitals”. *Clinical Infectious Diseases* 38.1 (2004): 78-85.

14. Honarm Hamidreza., *et al.* "Evaluation of a PCR assay to detect enterococcus faecalis in blood and determine glycopeptides resistance genes: van A and van B". *Iranian Journal of Medical Sciences* 37.3 (2012): 194.
15. Shahbazi Shahla., *et al.* "Distribution of extended-spectrum β -lactam, quinolone and carbapenem resistance genes, and genetic diversity among uropathogenic *Escherichia coli* isolates in Tehran, Iran". *Journal of Global Antimicrobial Resistance* 14 (2018): 118-125.
16. Noohi Nasrin and Malihe Talebi. "Diversity and Genetic Clonality of Vancomycin and Gentamicin Resistant Enterococci Isolated from Healthy Humans in Iran". *Journal of Medical Bacteriology* 8.1-2 (2019): 27-36.
17. Abat Cédric., *et al.* "Low level of resistance in enterococci isolated in four hospitals, Marseille, France". *Microbial Drug Resistance* 22.3 (2016): 218-222.
18. Suppli Morten., *et al.* "Mortality in enterococcal bloodstream infections increases with inappropriate antimicrobial therapy". *Clinical Microbiology and Infection* 17.7 (2011): 1078-1083.
19. Seyfi Mahnaz., *et al.* "Anti-microbial resistance of enterococci isolated from urinary tract infections in Iran". (2008): 185-190.
20. ShanMuKhappa., *et al.* "Isolation, Identification and Speciation of Enterococci by Conventional Method and Their Antibio-gram". *National Journal of Laboratory Medicine* 4.2 (2015): 1-6.
21. Mutnick Alan H., *et al.* "Geographic variations and trends in antimicrobial resistance among *Enterococcus faecalis* and *Enterococcus faecium* in the SENTRY Antimicrobial Surveillance Program (1997-2000)". *Diagnostic Microbiology and Infectious Disease* 46.1 (2003): 63-68.
22. Duez Colette., *et al.* "The penicillin resistance of *Enterococcus faecalis* JH2-2r results from an overproduction of the low-affinity penicillin-binding protein PBP4 and does not involve a *psr*-like gene. The GenBank accession numbers for the sequences reported in this paper are Y17797 for the 8.4 kb segment of pDML521; AJ290435 for *pbp4* of *E. faecalis* JH2-2; AJ276231 and AJ276232 for the *psr*-like gene of *E. faecalis* JH2-2 or JH2-2r, respectively". *Microbiology* 147.9 (2001): 2561-2569.
23. Jadhav R K., *et al.* "Bacterial urinary tract infections in canines and their management". *North-East Veterinarian* 11.2 (2011): 29-16.
24. Maschieto Andresa., *et al.* "Antimicrobial resistance of *Enterococcus* sp. isolated from the intestinal tract of patients from a university hospital in Brazil". *Memórias do Instituto Oswaldo Cruz* 99.7 (2004): 763-767.
25. Kaçmaz Birgül and Altan Aksoy. "Antimicrobial resistance of enterococci in Turkey". *International journal of Antimicrobial Agents* 25.6 (2005): 535-538.
26. Chajęcka-Wierzchowska., *et al.* "Virulence factors of *Enterococcus* spp. presented in food". *LWT* 75 (2017): 670-676.
27. M'hir Sana., *et al.* "Technological, functional and safety aspects of enterococci in fermented vegetable products: a mini-review". *Annals of Microbiology* 62.2 (2012): 469-481.
28. Golob Majda., *et al.* "Antimicrobial resistance and virulence genes in *Enterococcus faecium* and *Enterococcus faecalis* from humans and retail red meat". *BioMed research international* 2019 (2019).
29. Weng Poh Leng., *et al.* "High genetic diversity of *Enterococcus faecium* and *Enterococcus faecalis* clinical isolates by pulsed-field gel electrophoresis and multilocus sequence typing from a hospital in Malaysia". *BioMed research international* 2013 (2013).

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