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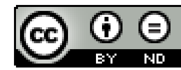

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*Original Article:*

# Clinico-Epidemiological Profile of VRE Enterococcus faecium in Shariati Hospital in Tehran

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**Abstract:** Background: Owing to restricted treatment options, Vancomycin-resistant Enterococci (VRE) was considered a prominent cause of nosocomial infections. This study was undertaken to evaluate the presence of Van-type and virulence determinants in the clinical isolates of E. faecium (Ent. faecium) in Shariati Hospital. Materials and Methods: A total of 150 Enterococcal isolates were surveyed. Antimicrobial susceptibility testing was performed by disc diffusion and E-test as well as the genotypic method. The presence of virulence factors, including hyaluronidase (hyl), gelatinase (gelE), aggregation substance (asa1), and Enterococci surface protein (ESP) were identified by Polymerase chain reaction (PCR). Results: Overall, 66.67 percent (80/120) of VRE Ent. faecium strains were confirmed by the PCR method. The maximum number of isolates was from urine specimens ($p < 0.05$) and blood samples. Among the 80 VRE Ent. faecium isolates, 76 isolates showed high-level resistance (MICs to Vancomycin 32 > µg/ml) and carried a VanA phenotype ($p < 0.05$). In all the isolates, asa1, gelE, and ESP genes were identified in 14% (17/5), 26/3% (21/80), and 45% (36/80), respectively. E. Ent. faecium carried ESP at a significantly higher frequency presented in VRE strains ($p < 0.001$). The prevalence of hly determinants in the E. faecium was 20% (16) ($P < 0.001$). Conclusion: We, in our hospital, are faced with a high rate of VRE Ent. faecium isolates with a VanA-positive phenotype. With increasing resistance of the VRE strains to linezolid, we will encounter a serious challenge in treating VRE patients in future years. An interesting finding from the present study is that the spreading rates of ESP and hly among Ent. faecium isolates are higher.
**Key Words:** Enterococcus faecium, Antibiotic resistance, Vancomycin-resistant enterococci, Virulence factors

## Introduction:

Enterococci is considered as the second most common nosocomial (hospital-acquired) infection; particularly endocarditis, ureteric infections, bacteremia, endocarditis, and meningitis (1, 2). Many species are responsible for human disease; however, Enterococcus faecium represent more than 90% of the clinical isolates (1). In recent years, Enterococcus faecium has dramatically increased and emerged as an important cause of the multidrug-resistant (MDR) Enterococcal infection (3). The acquired resistance to several important clinical antibiotics, such as the resistance to Vancomycin in the Enterococcus isolates, has been a particular concern (3). Moreover, the increasing resistance to Vancomycin, high-level penicillin-resistant (HLPR) and gentamicin-resistant, has recently emerged (4, 5). The emergence of resistance to quinupristin/dalfopristin, daptomycin, and linezolid as therapeutic and preventative options led to dramatic challenges in treating MDR enterococcal infections (5-7) . Five main types (VanA, B, D, E, and G) of Vancomycin-resistance have been described based on both the phenotypic and genotypic methods (7). The VanA-type is responsible for the high levels of inducible resistance to both vancomycin and teicoplanin, whereas the VanB-type, can only cause variable levels of resistance to Vancomycin (7). The VanC phenotype is characterized by low resistance to Vancomycin and teicoplanin susceptibility (7). Both Enterococcus faecalis and Enterococcus faecium are adaptable pathogens involving some host-specific lineages(8). Strains from human adapted clonal complexes (CCs) is a well-established hospital pathogen associated with outbreaks and characterized by resistance to various antibiotics, such as quinolones and ampicillin (ARE)(8). Also, it is associated with the presence of a putative pathogenicity island markers, comprising the enterococcal surface protein encoding gene (esp) and hyaluronidase gene (hyl), and the IS16 insertion element esp gene. Without prior knowledge of Van-genotype, an expensive drug is prescribed for treatment in hospitals. Therefore, determining the Van genotype and the rate of distribution in order to appropriately prescribe a treatment for the patients is essential. In addition, data of the virulence determinants, including hyaluronidase (hyl), gelatinase (gelE),

aggregation substance (asa1), and enterococcal surface protein (ESP) in the Enterococcus strains, in Ent. faecium is still limited (9). Therefore, the objective of the study was to evaluate the presence of Van-type and virulence determinants in clinical isolates of Ent. faecium in Shariati Hospital.

## Materials and Methods

### Strain collection

The clinical and epidemiological features of the eighty Enterococcal-infected patients were documented. This cross-sectional study was performed on 80 Ent. faecium isolates that were collected from patient samples urine (36), blood (18) and BAL10, wound (7), sputum (6), tissue (2), and abscess (1) in Shariati Hospital on 1 February 2016. All the patients in different parts of the hospital departments were sampled, including patients in the general intensive care unit (ICU), Bone Marrow unit, Hematopoietic Stem Cell Transplantation (HSCT), Nephrology unit, Outpatient, Digestive unit, Neonatal Intensive Care Unit (NICU), Rheumatology unit, Lung unit, Women Emergency, Glands unit, Urology unit, and the Oral and Maxillofacial Surgery unit (Table1). The isolates were confirmed and distinguished as Enterococcus spp by using routine microbiological methods; then, the PCR amplification of the D-alanine-D-alanine ligases determinants specific to *E. faecium* and *E. faecalis* were used to confirm the phenotypic characters (10).

### Antibiotic susceptibility testing

AST (Antimicrobial susceptibility testing) was performed by the modified Kirby Bauer disc diffusion method based on the CLSI guidelines against conventional antibiotics to determine the minimum inhibitory concentration (MIC). Determination of MIC of teicoplanin and Vancomycin (Sigma Aldrich, Germany) for the E. faecium isolates was performed using the E test (bioMerieux) method according to the CLSI guidelines (11). The results of MIC were interpreted according to CLSI guidelines (11).

**Table 1: PCR primers and products for the detection of virulence genes**

| Genes | | | Primer sequences (5'?3') | Product size (bp) | |
|---|---|---|---|---|---|
| Genotypic Detection of Enterococcus | *ddl E. faecalis* | | F=5'- ATCAAGTACAGTTAGTCTTTATTAG-3'<br>F=5'- ACGATTCAAAGCTAACTGAATCAGT-3' | 941 | (2) |
| | *ddl E. faecium* | | F=5' TTGAGGCAGACCAGATTGACG -3'<br>F=5'- TATGACAGCGACTCCGATTCC-3' | 658 | |
| Virulence genes | *Aggregation substance* | *asa1* | F:5'- GCACGCTATTACGAACTATGA -3'<br>R: 5- TAAGAAAGAACATCACCACGA -3' | 375 | (2,10) |
| | *Gelatinase* | *gelE* | F= 5'- TATGACAATGCTTTTTGGGAT -3'<br>R=5'- AGATGCACCCGAAATAATATA -3' | 688 | |
| | *Enterococcal surface protein* | *esp* | F= 5'- AGATTTCATCTTTGATTCTTGG -3'<br>R=5'- AATTGATTCTTTAGCATCTGG -3' | 510 | |
| | *Hyaluronidase* | *hyl* | F=5'- ACAGAAGAGCTGCAGGAAATG -3'<br>R=5'- GACTGACGTCCAAGTTTCCAA -3' | 276 | |

### DNA Extraction and PCR

Total DNA extraction was performed by the QIAamp DNA mini kits (QIAGEN, Germany). Extracting DNA from the fresh cultures was performed based on following the manufacturer's instructions. PCR assay was used for the detection of the Van A, Van C, and Van B genes in the VRE strains described by Kariyama et al. VanA amplification was performed with the primers VanA Forward: 5'- AATACTGTTTGGGGGTTGCTC-3' and VanA Reverse: 5' – CTTTTTCCGGCTCGACTTCCT- 3' to yield a 734-bp fragment, while the van was amplified with the primers VanB Forward: 5'- CATCGTCCCCGAATTTCAAA- 3' and van R 5'- GATGCGGAAGATACCGTGGCT- 3' to yield a 295-bp fragment, and Van C2/C3 F- 5' CGCAGGGACGGTGATTTT- 3' and C2/C3 Reverse: 5'- CGGGGAAGATGGCAGTAT- 3' to yield a 484-bp fragment. The presence of gelE, ESP, and asa1 genes specific for virulence determinants were confirmed by the PCR assay method as described by Vankerckhoven (2001)(12). Then, the PCR method with appropriate primers and cycling conditions was performed. The sequences of primers and annealing temperatures used in our study are presented in Table I.

### Statistical Analysis

All the statistical analyses were done by the SPSS (version 18) software, SPSS Inc.

### Enterococci Isolates

In this cross-sectional study, a total of 120 Ent. faecium isolates were collected from 13,100 samples over a period of one year from different clinical specimens by using biochemical methods. The rate of the infection was estimated to be 9.2%. Overall, 80 VRE Ent. faecium strains were confirmed by the PCR method. Of the 80 VRE isolates, the maximum number of isolates were from urine specimens 25 (45%), blood samples (22. 5%), and other samples with low numbers, including BAL, wound, sputum, tissue, and abscess (Fig. 1). Twenty-seven (33/7%) VRE isolates were associated with the age group of people under 50 years old. Therefore, in the present study, the presence of VRE Ent. faecium isolates in patients of 50 years or older shower significantly higher prevalence ($p < 0.05$). The rate of the prevalence of VRE isolates was equal across sexes (40 strains were related to males and 40 were related to females).

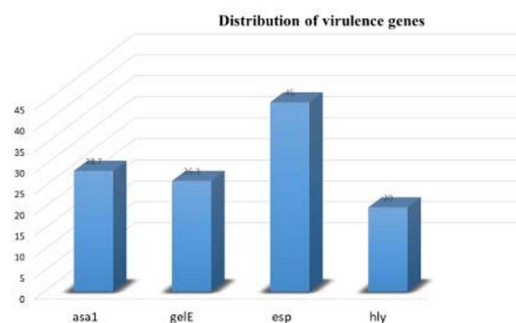


**Fig. 1. Distribution of virulence genes among VRE *E.faecium* isolates**

**Antimicrobial Susceptibility Testing**

VRE Ent. faecium isolates were tested for susceptibility to six antimicrobial agents using the disk diffusion method. The isolates revealed resistance to ampicillin (87%), gentamicin 56 (83/5%), linezolid 18 (22/5% intermediate), penicillin 78 (97/5%), Vancomycin (100%), and nitrofurantoin 4/34 (11/8%). Among the 80 VRE Ent. faecium isolates, 76 isolates showed high level resistance to (MICs to vancomycin 32 > µg/ml) carried a VanA phenotype while 4 isolates revealed that MIC Vancomycin (4-512 µg/ml) harbored VanB phenotype. None of the VRE isolates carried the VanB and the VanA phenotypes. Nitrofurantoin was used only for 34 urine isolates and 11/8% of all the urine isolates showed in vitro resistance to it. None of the Enterococcus isolates had high-level resistance to linezolid, but 22/5% intermediate resistance was observed in a number of isolates. The susceptibility patterns of Ent. faecium to antibiotics have been presented in Table 2.

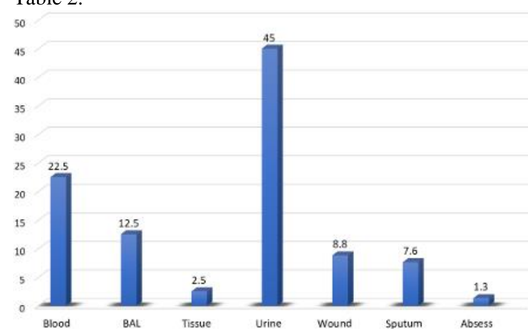


**Fig. 2. Comparative frequency of VRE E. faecium isolates among different sources.**

**Table 2: The susceptibility patterns of *E. faecium* to antibiotics**

| MDR pattern | Nit | P | GM/20 | AM | LZD | V | Department | Age | Sex | Specimen | Strain | Reception code |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| VRE | - | R | R | R | S | R | ICU nerves | 23 | F | Blood | Ent.Facium | 492704 |
| VRE | - | R | R | S | S | R | CCU Heart | 77 | M | BAL | Ent.Facium | 2195 |
| VRE | - | R | R | R | S | R | CCU Heart | 81 | M | BAL | Ent.Facium | 9675 |
| VRE | - | R | R | S | S | R | CCU Heart | 50 | M | BAL | Ent.Facium | 8202 |
| VRE | - | R | R | R | S | R | ICU Heart | 49 | F | Blood | Ent.Facium | 9938 |
| VRE | - | R | R | R | S | R | General Internal | 77 | M | Blood | Ent.Facium | 8158 |
| VRE | - | R | R | R | S | R | Glands (medical 2) | 97 | M | Tissue | Ent.Facium | 14089 |
| VRE | - | R | R | R | S | R | Surgery room | 55 | M | Tissue | Ent.Facium | 13782 |
| VRE | S | R | R | R | S | R | Emergency Clinic | 81 | M | Urine | Ent.Facium | 17716 |
| VRE | - | R | S | R | S | R | POST HSCT | 22 | M | BAL | Ent.Facium | 24617 |
| VRE | - | R | R | R | S | R | Glands (medical 2) | 47 | M | Wound | Ent.Facium | 24634 |
| VRE | - | R | R | S | S | R | Internal ICU | 55 | M | Sputum | Ent.Facium | 41340 |
| VRE | - | R | R | R | S | R | Rheumatology (medical 1) | 81 | F | Urine | Ent.Facium | 42295 |
| VRE | - | R | R | S | S | R | CCU Heart | 77 | M | Sputum | Ent.Faecalis | 9672 |
| VRE | - | R | R | R | S | R | POST HSCT | 18 | M | BAL | Ent.Facium | 52049 |
| VRE | S | R | R | R | I | R | ICU | 67 | M | Urine | Ent.Facium | 158651 |
| VRE | - | R | S | R | I | R | Oral and Maxillofacial Surgery | 57 | M | Wound | Ent.Facium | 157155 |
| VRE | S | R | R | R | S | R | Urology | 54 | M | Urine | Ent.Facium | 145513 |
| VRE | S | R | R | R | S | R | ICU nerves | 72 | M | Urine | Ent.Facium | 159316 |
| VRE | S | R | R | R | S | R | Neurology | 31 | F | Urine | Ent.Facium | 66085 |
| VRE | S | R | R | R | S | R | Rheumatology | 29 | F | Urine | Ent.Facium | 59106 |
| VRE | - | R | R | R | S | R | ICU | 64 | F | BAL | Ent.Facium | 72455 |
| VRE | - | R | R | R | S | R | Glands medical 2)) | 36 | M | Absess | Ent.Facium | 22898 |
| VRE | S | R | S | R | S | R | ICU General | 19 | M | Urine | Ent.Facium | 35937 |
| VRE | S | R | R | R | S | R | Lung | 82 | M | Urine | Ent.Facium | 35752 |
| VRE | - | R | R | R | S | R | ICU | 67 | F | Blood | Ent.Facium | 57964 |
| VRE | S | R | S | R | S | R | Neurology | 84 | M | Urine | Ent.Facium | 56970 |
| VRE | - | R | R | R | I | R | ICU | 82 | F | Blood | Ent.Facium | 115416 |
| VRE | - | R | R | R | S | R | Urology | 53 | M | Blood | Ent.Facium | 85528 |
| VRE | - | R | R | R | I | R | BMT3 | 5 | M | Blood | Ent.Facium | 154481 |
| VRE | S | R | R | R | S | R | Rheumatology (medical 1) | 29 | F | Urine | Ent.Facium | 172201 |
| VRE | S | R | R | R | S | R | POST HSCT | 33 | F | Urine | Ent.Facium | 155971 |
| VRE | - | R | R | R | S | R | Neurology surgery | 65 | F | Urine | Ent.Facium | 173014 |
| VRE | S | R | S | R | S |  | ICU (Heart) | 78 | M | Urine | Ent.Facium | 163852 |
| VRE | S | R | R | R | I | R | Neurology | 57 | M | Urine | Ent.Facium | 156823 |
| VRE | - | R | S | S | S | R | ICU | 21 | M | Wound | Ent.Facium | 87302 |
| VRE | - | R | R | R | I | R | Emergency Women | 37 | F | Urine | Ent.Facium | 173414 |
| VRE | S | R | S | R | S | R | ICU (Internal) | 29 | F | Urine | Ent.Facium | 164022 |
| VRE | - | R | R | R | S | R | ICU (Internal) | 84 | M | Blood | Ent.Facium | 266513 |
| VRE | - | R | R | R | S | R | Neurology | 85 | M | Sputum | Ent.Facium | 244545 |
| VRE | S | R | R | R | S | R | Lung | 58 | F | Urine | Ent.Facium | 273767 |
| VRE | - | R | R | R | I | R | ICU (General) | 31 | F | Wound | Ent.Facium | 273596 |
| VRE | - | R | R | S | S | R | Neurology | 20 | F | Blood | Ent.Facium | 249612 |
| VRE | S | R | R | R | I | R | ICU (General) | 78 | M | Urine | Ent.Facium | 449632 |
| VRE | - | R | R | R | S | R | ICU (General) | 82 | M | BAL | Ent.Facium | 268143 |

| VRE | - | R | R | R | I | R | General Internal | 76 | F | Blood | Ent.Facium | 248301 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| VRE | R | R | R | R | S | R | ICU | 51 | F | Wound | Ent.Facium | 266397 |
| VRE | - | R | S | R | S | R | ICU (General) | 50 | M | BAL | Ent.Facium | 244608 |
| VRE | - | R | R | S | I | R | General Internal | 61 | F | Wound | Ent.Facium | 262197 |
| VRE | - | R | R | R | S | R | ICU | 60 | M | Urine | Ent.Facium | 244042 |
| VRE | - | R | S | R | S | R | ICU | 73 | M | Blood | Ent.Facium | 262177 |
| VRE | S | R | R | S | S |  | Nephrology | 77 | F | Urine | Ent.Faecalis | 252925 |
| VRE | S | R | S | R | S | R | Blood | 25 | F | Urine | Ent.Faecalis | 55966 |
| VRE | S | R | R | R | S | R | ICU nerves | 66 | F | Urine | Ent.Facium | 128910 |
| VRE |  | R | S | S | S | R | Lung | 36 | M | BAL | Ent.Faecalis | 123966 |
| VRE | S | R | R | S | S | R | Rheumatology (medical 1) | 32 | F | Urine | Ent.Facium | 138762 |
| VRE | S | R | R | R | I | R | ICU | 67 | F | Urine | Ent.Facium | 124097 |
| VRE | S | R | S | R | I | R | Lung | 78 | F | Urine | Ent.Facium | 389700 |
| VRE | - | S | R | S | S | R | ICU | 67 | F | Sputum | Ent.Faecalis | 124339 |
| VRE | - | R | S | R | I | R | Internal ICU | 69 | F | Blood | Ent.Facium | 7078 |
| VRE | - | R | R | R | S | R | Digestive (medical 2) | 54 | F | Blood | Ent.Facium | 53783 |
| VRE | - | S | R | S | S | R | Nephrology | 69 | F | Sputum | Ent.Facium | 48138 |
| VRE | - | R | R | R | I | R | Blood | 35 | F | Blood | Ent.Facium | 110840 |
| VRE | S | R | S | R | S | R | ICU (General) | 53 | F | Urine | Ent.Facium | 128391 |
| VRE | - | R | R | R | S | R | Rheumatology | 60 | M | BAL | Ent.Facium | 23182 |
| VRE | - | R | R | R | S | R | Internal ICU | 39 | M | Blood | Ent.Facium | 95966 |
| VRE | R | R | S | R | S | R | Digestive (medical 2) | 90 | F | Urine | Ent.Facium | 17472 |
| VRE | S | R | R | R | S | R | NICU | 55 days | F | Urine | Ent.Facium | 11346 |
| VRE | - | R | S | R | S | R | Nephrology | 75 | F | Sputum | Ent.Facium | 12684 |
| VRE | S | R | R | R | S | R | OP | 51 | M | Urine | Ent.Facium | 61017 |
| VRE | R | R | R | R | I | R | Blood | 41 | M | Urine | Ent.Facium | 391089 |
| VRE | R | R | R | R | I | R | Internal ICU | 70 | F | Urine | Ent.Facium | 428749 |
| VRE | S | R | R | R | I | R | Nephrology | 77 | M | urine | Ent.Facium | 25766 |
| VRE | - | R | R | R | S | R | General Internal | 80 | F | Blood | Ent.Facium | 398475 |
| VRE | S | R | R | R | I | R | Nephrology | 62 | F | Urine | Ent.Facium | 42043 |
| VRE | - | R | R | R | S | R | ICU (General) | 56 | F | Blood | Ent.Facium | 6062 |
| VRE | - | R | R | R | S | R | ICU (General) | 54 | M | Wound | Ent.Facium | 103787 |
| VRE | - | R | R | R | S | R | POST HSCT | 11 | M | Blood | Ent.Facium | 109402 |
| VRE | S | R | R | R | S | R | Neurology | 48 | F | Urine | Ent.Facium | 74756 |
| VRE | S | R | GM/20 | R | S | R | BMT3 | 8 months | F | Urine | Ent.Facium | 95185 |

**Prevalence of Virulence Genes in *E. faecium***

In 80 of the VRE isolates, asa1, gelE, and ESP genes were identified in 14(17/5%), 26/3 ( 21/80%), and 45 (36/80%), respectively. Moreover, in Ent. faecium strains, the ESP gene was the most prevalent factor and the asa1 gene has a low number, followed by the hyl gene. E. faecium carried ESP at a significantly higher frequency presented in the VRE strains ($p < 0.001$). The prevalence of the hly determinants in the E. faecium was 16 (20%) ($P < 0.001$). The presence of the asa1 gene was also significant in the VRE strains ($p < 0.001$) as they were more commonly found in the VRE strains.

**Results of Statistical Analysis**

All the analyses were performed with the SPSS software (version 18.0) (USA). A chi-squared test was performed and $P < 0.05$ was considered statistically significant.

**Discussion**

Vancomycin-resistant Enterococci were introduced since 1986. These microorganisms were first detected in the hospital in London (12). The urinary tract is the most common site of Enterococcal infections. Enterococci can, however, cause serious infections such as gallbladder inflammation, bile duct inflammation, peritonitis, septicemia, endocarditis, and meningitis (13, 14). Enterococci in the incidence of the nosocomial infection in the past two decades have gained the third place, followed by Escherichia coli and Staphylococcus aureus (14). Enterococci are responsible for 10% of hospital-acquired infections in the USA (14). In urinary tract infections, Enterococci are constantly the second or the third most prevalent pathogens, bacteremia infections, wounds, and infections in hospitals (14). This pathogen accounted for approximately 16% of nosocomial urinary tract infections (14).

In this study of 150 Enterococcus strains, 120 (80%) were identified as *Ent. faecium* isolates. To disagree with our study, *Enterococcal faecalis* is the most common isolate of Enterococci in most of the studies inside and outside the country in the clinical samples (15-17) . Studies by Ajay et al. and Sreeja et al. in India, in 2012, showed that the prevalence of the *Enterococcus faecalis*strains were 55.5% and 76%, respectively (17). In other works by Fisher and Phillips in 2009 (18) and Ukropina-Mihajlovic in 2012 (19) reported that the situation of Enterococcus faecalis were high (3). In recent years, an increase in the rate of *Enterococcus faecium* compared to *Ent. faecium* is observed. In our study, the frequency of Vancomycin-resistance Enterococci was 53/3% (80/150). Dissimilar to our study, Deshpande et al. showed the prevalence of Vancomycin-resistance as 19.6% (4, 20-22) . In disagreement with our study, Bhatt et al. (23) and all the other studies from India have stated a prevalence of approximately 10%.

Exposed to different antibiotics, including Vancomycin, cephalosporin, augmenting Vancomycin's selective pressure can be one of the main reasons for a high prevalence of Vancomycin-resistance among Enterococci (24). In a study, Bhatt et al. reported that only 13 isolates showed a high-level of resistance to Vancomycin in which one gene was extant in these 13 isolates (24). Among the VRE strains, the VanA phenotype is the most common genotype, particularly in isolates with a high-level resistance to both Vancomycin and teicoplanin. Similar to our study, Praharaj et al. presented that

the VanA phenotype was detected in a high number of all the VRE isolates (87.5%) (24). According to the typing results of the genotypes, among the VRE strains found in Shariati Hospital in Tehran, genotype VanB was the second most common genotype. In agreement with our results, Nasaj et al. showed that two VRE isolates (3.3%) carried the VanB gene (10). Therefore, in Tehran hospitals, conventional antibiotics cannot be considered as suitable treatments for Vancomycin-resistant strains. However, there was no resistance to a new antibiotic, such as linezolid, which could be an appropriate therapeutic choice. New antibiotics such as linezolid, tigecycline, and daptomycin can be used as alternatives for the treatment of Enterococcal infections with multiple-drug resistance.

It should be pointed out that among the various antimicrobials available and assessed for the treatment of serious infections with Vancomycin-resistant enterococci, linezolid is very effective in our hospitals. In agreeing with our study, in recent years, increasing high-level resistance to aminoglycosides, penicillin, and ampicillin has been particularly revealed in Vancomycin-resistant Ent. faecium isolates (9, 25). With respect to the results in our hospital, linezolid was prescribed as a first-choice in patients infected by VRE Enterococci while we faced with increases in the prevalence of intermediate linezolid resistance. Recently, outbreaks related to the VRE Ent. faecium infection in patients without prior exposure to linezolid have been described by Rahim et al. (26). In addition, the prevalence of multiple clones of linezolid-resistant E.f from the other parts of the world have been reported (27). In this situation in our hospitals, the evaluation all the VRE isolates for susceptibility, following on to appropriate infection-control measures, and to emphasize the significance of using linezolid with caution is necessary.

The ESP determinants contribute to the biofilm formation and the interface with primary surfaces (4, 28) . In disagreement with our study, Vankerckhoven et al. (12) described that the gelE and asa1 genes in the European E. faecium isolates were not identified. This results are approximately agreed on in the reports that are mentioned in the presence of one or more of these genes by other researchers (12, 29, 30) . Consenting to our study, Comerlato et al. (in Brazil) indicated that asa1, gelE, and ESP genes were detected in 38%, 60%, and 76%, respectively, with all the isolates, respectively (2). This difference can only be due to the isolates of *Ent. faecium* that have been studied in our study; in other research, however, the prevalence of both Enterococcal groups had been investigated. The ESP determinants supported the primary attachment and the biofilm formation in the bacteria to the urinary tract and polyvinyl chloride plastic (28). Our findings appear to be well-supported by Arshadi et al. (31), who showed a high number of ESP-positive *Ent. faecium* compared with asa1 and gelE genes with low frequencies. The high prevalence of the ESP gene in *Ent. faecium* indicated the important role of the gene in the virulence process (32).

The virulence gene ESP is related to hospital outbreaks throughout the world and a significant feature of clonal-complex 17 (CC17) (33). In the present study, the pattern of antimicrobial resistance in the Enterococcus strains was not related to the presence or the absence of virulence genes ($P < 0.05$). Hyl (hyaluronidase), encoded by chromosomal DNA, has been associated with significant tissue damage by degradative enzyme activity (18, 34). We detect the hyl gene among 16 (20%) the VRE Ent. faecium isolates. The hyl gene was identified in 16 (20%) of the 80 Ent. faecium isolates collected in Shariati Hospital, while our finding is in contrast to the work of Rice et al. (3%) (35). Moreover, in contrast to our study prevalence of the hyl gene in VREEnt. faecium isolates, it was more widespread among the United Kingdom isolates (71%) (36).

**Conclusion**

This study illustrates the Ent. faecium isolates had a high level frequency in our hospital. Furthermore, in the Shariati hospital, we were faced with high rate of VRE isolates in Ent. faecium, with VanA-positive Ent. faecium isolates. With increasing resistance of the VRE strains to linezolid, we will encounter serious challenges in treating VRE patients in the future. In addition, an interesting finding from the present study was the higher spreading rates of ESP and hly among *Ent. faecium* spp. In such cases, the use of policy and regular efficient surveillance in order to control of VRE Ent. faecium strain in our hospitals is important during emergencies.

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**References:**

1. Conte JE. Manual of antibiotics and infectious diseases: treatment and prevention: Lippincott Williams & Wilkins; 2002.
2. Comerlato CB, Resende MCCd, Caierão J, d'Azevedo PA. Presence of virulence factors in Enterococcus faecalis and Enterococcus faecium susceptible and resistant to vancomycin. *Memórias do Instituto Oswaldo Cruz.* 2013;108(5):590-5.
3. Higuita NIA, Huycke MM. Enterococcal disease, epidemiology, and implications for treatment. 2014.
4. Emaneini M, Khoramian B, Jabalameli F et al. Prevalence of high-level gentamicin-resistant *Enterococcus faecalis* and *Enterococcus faecium* in an Iranian hospital. *Journal of Preventive Medicine and Hygiene*. 2016;57(4):E197.
5. Adhikari L. High-level aminoglycoside resistance and reduced susceptibility to vancomycin in nosocomial enterococci. *Journal of Global Infectious Diseases*. 2010;2(3):231.
6. Arias CA, Contreras GA, Murray BE. Management of multidrug-resistant enterococcal infections. *Clinical Microbiology and Infection.* 2010;16(6):555-62.
7. Courvalin P. Vancomycin resistance in gram-positive cocci. *Clinical Infectious Diseases.* 2006;42(Supplement_1):S25-S34.
8. Billström H, Top J, Edlund C, Lund B. Frequent occurrence of multidrug-resistant CC17 Enterococcus faecium among clinical isolates in Sweden. *Journal of Applied Microbiology.* 2010;108(5):1810-6.
9. Eliopoulos GM, Meka VG, Gold HS. Antimicrobial resistance to linezolid. *Clinical Infectious Diseases.* 2004;39(7):1010-5.
10. Nasaj M, Mousavi SM, Hosseini SM, Arabestani MR. Prevalence of virulence factors and vancomycin-resistant genes among *Enterococcus faecalis* and *E. faecium* isolated from clinical specimens. *Iranian Journal of Public Health.* 2016;45(6):806.
11. CLSI. M100-S25 performance standards for antimicrobial susceptibility testing; Twenty-fifth informational supplement; 2015.
12. Vankerckhoven V, Van Autgaerden T, Vael C, Lammens C, Chapelle S, Rossi R, et al. Development of a multiplex PCR for the detection of asa1, gelE, cylA, esp, and hyl genes in enterococci and survey for virulence determinants among European hospital isolates of *Enterococcus faecium*. *Journal of Clinical Microbiology.* 2004;42(10):4473-9.

13. Kak V, Chow JW. Acquired antibiotic resistances in enterococci. The enterococci: American Society of Microbiology; 2002. p. 355-83.
14. MSDS Online. Enterococcus faecalis and faecium. Pathogen Safety Data Sheet - Infectious Substances. Available at: https://www.msdsonline.com/resources/msds-resources/free-safety-data-sheet-index/enterococcus-faecalis-and-faecium/.
15. Peciuliene V, Balciuniene I, Eriksen HM, Haapasalo M. Isolation of Enterococcus faecalis in previously root-filled canals in a Lithuanian population. *Journal of Endodontics.* 2000;26(10):593-5.
16. Vidyalakshmi P, Gopalakrishnan R, Ramasubramanian V, Ghafur KA, Nambi PS, Thirunarayana M. Clinical, epidemiological, and microbiological profile of patients with vancomycin-resistant Enterococci from a Tertiary Care Hospital. *Journal of Global Infectious Diseases.* 2012;4(2):137.
17. Sreeja S, Sreenivasa Babu P, Prathab A. The prevalence and the characterization of the enterococcus species from various clinical samples in a tertiary care hospital. *Journal of Clinical and Diagnostic Research: JCDR.* 2012;6(9):1486.
18. Fisher K, Phillips C. The ecology, epidemiology and virulence of Enterococcus. *Microbiology.* 2009;155(6):1749-57.
19. Mira M-U, Deana M, Zora J, Vera G, Biljana M, Biljana R. Prevalence of different enterococcal species isolated from blood and their susceptibility to antimicrobial drugs in Vojvodina, Serbia, 2011-2013. *African Journal of Microbiology Research.* 2014;8(8):819-24.
20. Willems RJ, Top J, Marga van Santen D et al. Global spread of vancomycin-resistant Enterococcus faecium from distinct nosocomial genetic complex. *Emerging Infectious Diseases.* 2005;11(6):821.
21. Werner G, Klare I, Fleige C, Witte W. Increasing rates of vancomycin resistance among Enterococcus faecium isolated from German hospitals between 2004 and 2006 are due to wide clonal dissemination of vancomycin-resistant enterococci and horizontal spread of vanA clusters. *International Journal of Medical Microbiology.* 2008;298(5-6):515-27.
22. Treitman AN, Yarnold PR, Warren J, Noskin GA. Emerging incidence of Enterococcus faecium among hospital isolates (1993 to 2002). *Journal of Clinical Microbiology.* 2005;43(1):462-3.
23. Bhatt P, Sahni A, Praharaj A, Grover N, Kumar M, Chaudhari C, et al. Detection of glycopeptide resistance genes in enterococci by multiplex PCR. *Medical Journal Armed Forces India.* 2015;71(1):43-7.
24. Praharaj I, Sujatha S, Parija SC. Phenotypic & genotypic characterization of vancomycin resistant Enterococcus isolates from clinical specimens. *The Indian Journal of Medical Research.* 2013;138(4):549.
25. Eliopoulos GM, Gold H. Vancomycin-resistant enterococci: mechanisms and clinical observations. *Clinical Infectious Diseases.* 2001;33(2):210-9.
26. Rahim S, Pillai SK, Gold HS, Venkataraman L, Inglima K, Press RA. Linezolid-resistant, vancomycin-resistant *Enterococcus faecium* infection in patients without prior exposure to linezolid. *Clinical Infectious Diseases.* 2003;36(11):e146-e8.
27. Ntokou E, Stathopoulos C, Kristo I, Dimitroulia E, Labrou M, Vasdeki A, et al. Intensive care unit dissemination of multiple clones of linezolid-resistant *Enterococcus faecalis* and *Enterococcus faecium. Journal of Antimicrobial Chemotherapy.* 2012;67(8):1819-23.
28. Toledo-Arana A, Valle J, Solano C et al. The enterococcal surface protein, Esp, is involved in Enterococcus faecalis biofilm formation. *Applied and Environmental Microbiology.* 2001;67(10):4538-45.
29. Eaton TJ, Gasson MJ. Molecular screening of Enterococcus virulence determinants and potential for genetic exchange between food and medical isolates. *Applied and Environmental Microbiology.* 2001;67(4):1628-35.
30. Elsner H-A, Sobottka I, Mack D, Laufs R, Claussen M, Wirth R. Virulence factors of Enterococcus faecalis and Enterococcus faecium blood culture isolates. *European Journal of Clinical Microbiology and Infectious Diseases.* 2000;19(1):39-42.
31. Arshadi M, Douraghi M, Shokoohizadeh L, Moosavian SM, Pourmand MR. High prevalence of diverse vancomycin resistance Enterococcus faecium isolates in clinical and environmental sources in ICU wards in southwest of Iran. *Microbial Pathogenesis.* 2017;111:212-7.
32. Willems RJ, Bonten MJ. Glycopeptide-resistant enterococci: deciphering virulence, resistance and epidemicity. *Current Opinion in Infectious Diseases.* 2007;20(4):384-90.
33. Soares RO, Fedi AC, Reiter KC, Caierão J, d'Azevedo PA. Correlation between biofilm formation and gelE, esp, and agg genes in Enterococcus spp. clinical isolates. *Virulence.* 2014;5(5):634-7.
34. Kayaoglu G, Ørstavik D. Virulence factors of Enterococcus faecalis: relationship to endodontic disease. *Critical Reviews in Oral Biology & Medicine.* 2004;15(5):308-20.
35. Rice LB, Carias L, Rudin S, Vael C, Goossens H, Konstabel C, et al. A potential virulence gene, hyl EEnt. faecium, predominates in Enterococcus faecium of clinical origin. *The Journal of Infectious Diseases.* 2003;187(3):508-12.
36. Dutka-Malen S, Evers S, Courvalin P. Detection of glycopeptide resistance genotypes and identification to the species level of clinically relevant enterococci by PCR. *Journal of Clinical Microbiology.* 1995;33(1):24-7.