The Frequency of Class1 and 2 and 3 Integrons in Vancomycin-resistant and Aminoglycoside Resistance Enterococcus Strains Isolated From Burn Patients in Southwest Iran

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Abstract

Background: This study aimed to detect the frequency of class1 and 2 and 3 integrons in vancomycin-resistant enterococcus (VRE) and aminoglycoside resistance strains isolated from burn patients in Taleghani burn hospital, Ahvaz

Results: Out of the 129 Enterococcus species 42% were E. faecalis and 57% isolated were E. faecium. 34% were resistant to vancomycin. In the E. faecium isolates, 21% were XDR and 67%) were MDR. In the E. faecalis isolates, 74% were MDR isolates. Among 129 Enterococci spp. Isolates 45% E. faecium and 20% E. faecalis isolates were positive for the presence of vanA genes. In addition, 20% E. faecium and 0.04% E. faecalis isolates were positive for the presence of vanB genes. Thirty-one E Enterococci spp including E. faecium 79% and E. faecium 58% isolates carried int 1. In VRE E. faecalis 57.1% isolates positive for the presence of int 1and 57.1% isolates positive for the presence of int 1.

Conclusion: we showed a detailed resistance pattern of clinically isolated Enterococci species to common antibiotics. Accordingly, the results confirmed the association between simultaneous resistance to vancomycin. These results can guide antibiotic prescriptions against Enterococci infections.

Introduction

Enterococci are normal bacteria in the human gastrointestinal tract and are known as intestinal cocci and are now considered important human pathogens that can cause urinary tract infections, bacteremia, and wound infections (1). These bacteria are highly resistant to antibacterial agents and are the second leading cause of urinary tract infections and wound infections and the third leading cause of nosocomial infections (2).

Enterococci, especially Enterococcus faecium, have shown significant resistance to a wide range of antibiotics, especially beta-lactams, glycopeptides, and aminoglycosides. Multidrug resistance (MDR) is also clinically important today (3, 4). In recent decades, there has been a significant increase in the incidence of acquired resistance to several antibiotics, including glycopeptide antibiotics such as vancomycin known as vancomycin-resistant enterococcus enterococci (VRE) in hospitals and now they are a health problem in the world (5). Vancomycin resistance is transmitted by transposon or plasmid. At present, six glycopeptide resistance phenotypes including vanA, B, C, D, E, G have been identified in enterococci. vanA and vanB are the most common and important phenotypes and cause resistance to several classes of antibiotics and are clinically important (6). Aminoglycosides are potent bacteriostatic agents that inhibit protein synthesis by binding to the ribosomal 30s unit and are often used in combination with beta-lactam antibiotics or a glycopeptide, especially to treat enterococcal infections (7). The main mechanism of resistance to aminoglycosides in enterococci is the inactivation of the drug by cellular aminoglycoside-modifying enzymes with plasmid or transposon genes. Several genes including aph-Ic, aph-Ib, aph-Ia, aph-Id, aph – IIIa, and ant-la cause enterococcal resistance to aminoglycoside(4). The most common of these in enterococci is the enzyme nucleotidyl transferase, which is encoded by the ant-la gene, and the resistance is dependent on the enzyme phosphotransferase, which is encoded by the aph – IIIa genes (4). Integrons are a new group of transmissible genetic elements that can transmit antibiotic resistance genes and are classified into four classes. Class 1 integrons are the most common. Class 1 integrons contain intI and attI genes that encode sulfonamide (Sull) resistance and disinfectants. Class 2 integrons are similar to class 1 and contain the integrase (intI2) gene. Class 3 integrons contain the intI3 gene and are structurally similar to class 1 integrons and contain the metallobetalactamase genes (8, 9).

As the incidence of VRE in hospitals increases, the rate of enterococcal isolation in burn patients whose first immune defense barrier has been removed has increased. In Iran, as in other countries, infections caused by vancomycin-resistant enterococci are increasing and are associated with mortality, especially in people with weakened immune systems. Epidemiological studies and the distribution of pathogenic bacteria are important to control the spread of drug resistance in different geographical areas. (10) Considering the importance and increase of enterococci resistance to vancomycin and aminoglycosides in hospitals, especially in burn patients, and also considering the important role of integrons in antimicrobial resistance and considering that a study has been conducted to study integrons on enterococci in southwest
Iran. In this study, the frequency of class1 and 2 and 3 integrons in vancomycin-resistant enterococcus and aminoglycoside resistance strains isolated from burn patients in southwest Iran were investigated.

**Materials And Methods**

**Bacterial isolates**

From September 2019 to June 2020, a total of 170 suspected enterococci isolates were collected from burn patients in Taleghani burn hospital, Ahvaz, southwest Iran.

All isolates were confirmed as Enterococcus spp. by Gram stain, growth in 6.5% NaCl, and bile and esculin hydrolysis. Following conventional test, for definitive identification of enterococci spp we used identification at the universal primer of the 16S rDNA gene. (11, 12)

**Antimicrobial susceptibility**

Antibiotic susceptibility testing was performed for 11 drugs covering all 11 antimicrobial categories comprising penicillin, aminoglycosides, ansamycins, fluoroquinolones, folate pathway inhibitors, tetracyclines, glycopeptides, macrolides and carbapenems were determined using the disc diffusion susceptibility test according to clinical and laboratory standards institute (CLSI) guidelines. Commercial antibiotic discs of rifampin (5 µg), linezolid (30 µg), ciprofloxacin (5 µg), tetracycline (10 µg), gentamycin (10 µg), erythromycin (15 µg), trimethoprim/sulfamethoxazole (1.25/23.75 µg), (MAST Diagnostics, Merseyside UK) were used in disc diffusion test. (13, 14)

**Screening for vancomycin resistance**

Resistance to vancomycin (MAST Diagnostics) was prepared by Mueller–Hinton agar (Merck, Germany) containing vancomycin 6 µg/mL and 4% NaCl. The plates were incubated at 37°C for 24 hours according to CLSI guidelines. Any visible growth after 24 hours was considered vancomycin resistant. (13)

**Molecular identification**

**DNA extraction and PCR amplification**

DNA was extracted from colonies of enterococci isolates by simple boiling method as described elsewhere (12). In brief, a few colonies were removed from fresh overnight culture on Muller- Hinton agar plates and dissolved in 500 µl of TE buffer, boiled for 10 min and placed at -20°C for 5 min. After centrifugation at 14000 × g for 10 min, the supernatant was used as a template for PCR.

**Detection of vanA and vanB, aac (6 ’) - aph (2 ’), int 1, int 2 genes**

PCR amplification was performed on extracted DNAs for the detection of int1, and int2, genes using the set of primers described previously explained by Haghi *et al* (15). PCR mix was prepared in a final volume of 25 µl containing 10X PCR Buffer, MgCl2 50 mM, dNTPs 10 mM, each primer 10 µM, Taq DNA Polymerase 5 U/µl, and 5 µl of extracted DNA. The amplification was performed in a thermocycler (Eppendorf, Germany), with following program: initial denaturation at 94°C for 5 min, 30 cycles of denaturation at 94°C for 1 min, annealing at 59°C for 1 min, extension at 72°C for 1 min and a final extension at 72°C for 5 min. P. aeruginosa ATCC 27853 was used as positive control. The PCR products were separated by electrophoresis on a 1.5% agarose gel containing 0.5 µg/ml ethidium bromide. The bands were visualized under UV light using a gel documentation system (Proteinsimple, USA). Descriptive statistics including frequencies, cross-tabulation of microbiological, clinical and demographic data were analyzed using SPSS statistical software (version 22.0). The Chi square test, was used in a univariate analysis to assess the differences between two groups of isolates (integron positive and integron negative) with antibiotic resistance. p values less than 0.05 were considered statistically significant (4, 11).
Identification of bacteria with amplification of gene encoding 16S rDNA

For definitive identification at the species level, an approximately 1500 bp fragment of the 16S rDNA gene was amplified and sequenced by two specific primers 27F (5′- AGA GTC CAT CMT GGC TCA A-3′) and 1525 (5′-AAG GGG AGG TGW TCC ARC G-3′) as previously described.15,16 The composition of PCR mixture was: 500 mM KCl, 200 mM Tris-HCl (PH = 8.4), 1.5 mM MgCl2, 0.2 Mm dNTPs, 0.4 µM each primer, 1 unit of Taq DNA polymerase, DW 30.3 µL, DNA Template 81 ng/ µL. The final volume was 50 µL. The PCR condition was initial denaturation one cycle of 95°C for 2 mines, followed by 30 cycles of 95°C for 30 s, annealing of 53.5°C for 30 s, an extension of 72°C for 1.30 mines and the final extension at 72°C for 10 mines. PCR amplifications for studied genes were conducted on a thermal cycler 5530 (Eppendorf master, Germany). The amplified PCR products of the 16S rDNA gene for each isolate were puried and the sequences of the products were determined using an ABI PRISM_ 7700 Sequence Detection System (Applied Biosystems, Foster City, CA, USA) according to the standard protocol of the supplier. The sequences of the 16S rDNA gene for each isolate were aligned separately and compared with all existing relevant sequences of recovered from the GenBank database using the JPhydit program.16 Percentages of similarity between sequences of each gene were determined by comparing sequences to an in-house database of 16S rDNA sequences.

Statistical Analysis

Qualitative variables presented as frequencies with percentages. Categorical variables were compared using the Fisher's exact test. All tests were two-tailed, and results with P values less than 0.05 were considered statistically significant. All data preparation and statistical analyses were conducted by using SPSS 22.

Results

Patient demographics

In the present study, From September 2019 to June 2020, a total of 170 specimens suspected to Enterococcus species were collected from different samples such as (urine, blood, Wound, secretory-nose and secretory-ear) of burn hospital. For definitive identification, all 170 isolates were subjected to 16S rDNA gene sequencing. The results of 16S rDNA gene sequence analysis revealed that 129 isolates showed more than 99% homology with relevant Enterococcus species. Out of the 129 Enterococcus species, (n = 55/129, 42%) were E. faecalis and (n = 74/129, 57%) isolated were E. faecium. In the study group, (n = 60/129, 46%) of the patients were women and (n = 69/129, 53%) were men. The mean age of the patients included in the study was 37.06 years and the mean ± standard deviation (SD) was 12.58 (Table 1). The prevalence rate of different specimens was (n = 14/129, 28%) wound, (n = 15/129, 30%) urine, song, secretory nose and secretory ear (n = 1/129) in clinical samples, respectively.

Table 2 shows the total distribution of E.faecalis and E.faecium in various types of clinical specimens in hospital.

Antimicrobial susceptibility

Out of the 129 clinical Enterococcus isolates (n = 28/126, 34%) were resistant to vancomycin by disc diffusion method. Out of the 28 clinical Enterococcus Isolates, (n = 17/28, 60%) were E. faecium and (n = 11/28, 39%) were E. faecalis. (Fig. 1) The prevalence rate of VRE in different specimens was (n = 13/50, 26%) blood, (n = 14/50, 28%) wound, (n = 15/50, 30%) urine, (n = 4/50, 8%) synovial fluid and (n = 4/50, 6%) abscesses in clinical samples. In antibiogram resistant of E. faecalis results shown a high rate of resistance was detected against erythromycin (n = 31/ 24%), gentamicin (n = 23/40%), penicillin (n = 23/17%). The result of antibiogram rate resistance in E. faecalis was shown in Table 3. In antibiogram resistant of E. faecium results showed a high rate of resistance was detected against penicillin (n = 33/ 25%), gentamicin (n = 37/38%), ciprofloxacin (n = 55/42%), and tetracycline (n = 32/50,64%), trimethoprim-sulfamethoxazole (n = 50,38%) antibiotic agents.
The result of antibiogram rate resistance in *E. faecium* was show in Table 4. In the *E. faecium* isolates, (n = 16/74, 21%) were XDR and (n = 50/74, 67%) were MDR. In the *E. faecalis* isolates, (n = 1/55) were XDR and (n = 41/55, 74%) were MDR isolates.

**Distribution of vancomycin and aminoglycoside resistance genes**

Among 129 *Enterococci* spp. isolates, (n = 59/129, 45%) *E. faecium* and (n = 27/129, 20%) *E. faecalis* isolates were positive for the presence of *vanA* genes. Among 129 *Enterococci* spp. isolates, (n = 21/129, 20%) *E. faecium* and (n = 6/129, 0.04%) *E. faecalis* isolates were positive for the presence of *vanB* genes. In VRE *E. faecium* (n = 10/28, 57.1%) isolates positive for the presence of *vanA* and (n = 3/28, 57.1%) isolates positive for the presence of *vanB*. In VRE *E. faecalis* (n = 4/28, 57.1%) isolates positive for the presence of *vanA* and (n = 6/28, 57.1%) isolates positive for the presence of *vanB*. Eighty-five *Enterococci* spp including *E. faecium* (n = 46/129, 79%) and *E. faecium* (n = 39/129, 58%) isolates carried *aac (6)*. In VRE *E. faecalis* (n = 12/28, 57.1%) isolates positive for the presence of aac (6') and (n = 16/28, 57.1%) isolates positive for the presence of *vanA* and (n = 4/28, 57.1%) isolates positive for the presence of *vanB*. The distribution of aminoglycoside resistance genes (ARGs) is presented in Table 1.

**Distribution of int of class 1 and 2 and 3 integrons in vancomycin and aminoglycoside resistance genes in enterococcus spp**

Thirty-one *Enterococci* spp including *E. faecium* (n = 16/129, 79%) and *E. faecium* (n = 15/129, 58%) isolates carried *int 1*.

In VRE *E. faecalis* (n = 3/28, 57.1%) isolates positive for the presence of *int 1* and (n = 10/28, 57.1%) isolates positive for the presence of *int 1*. Ten *Enterococci* spp including *E. faecium* (n = 10/28, 57.1%) isolates carried *int 2*. No VRE *E. faecalis* isolates positive for the presence of *int 2*.

In Table 1 and Table, the relationship between *Enterococci* spp strains comprising *int 1* and *int 2* antibiotic resistance are presented, some susceptible strains contained the gene as well. (Table 5)

**Discussion**

In recent years, due to excessive consumption of antibiotics, resistance to common antibiotics has escalated rapidly. Vancomycin-resistant enterococcus (VRE) is the second most frequent cause of nosocomial infections in the USA. (17) The prevalence of this bacterium in Iran is estimated to be 9.4%, particularly among culture-positive cases for Enterococcus species (18), while in developed countries such as Germany, the United Kingdom, and Italy, it has been demonstrated to be 11.2%, 8.5–12.5%, and 9%, respectively (19–23).

According to clinical studies, many hospital-associated strains that are resistant to vancomycin also show resistance to aminoglycosides. Therefore, the specific and accurate identification and determination of *Enterococcus* species and their antibiotic resistance pattern is essential to provide an effective treatment protocol, to choose the right drug for treating infection and to avoid transfer of vancomycin-resistant plasmid from Enterococcus to main pathogen bacteria and other Enterococcus strains (24). Accordingly, in the current study, 42% of samples were infected with *E. faecalis* and 57% with *E. faecium*.

Vancomycin is as an effective antibiotic against multidrug-resistant organisms, including VRE. Nonetheless, in this study, a low rate of vancomycin was observed. Our results revealed that the resistance rates of *E. faecalis* isolates to cell wall-active antibiotics, vancomycin and teicoplanin, were 9% and 0.03%, whereas those of *E. faecium* isolates were 2% and 17%, respectively, which are lower than the rates reported in other investigations (25, 26). Our findings also showed that the majority of *E. faecalis* and *E. faecium* isolates were resistant to erythromycin.
Similar to our study, there have been extensive surveys in Iran and other countries (27–29). In a study conducted by Shahraki et al. (2017), 182 samples were collected from the southeast of Iran. Among the samples, 63 and 22 cases were infected by *E. faecalis* and *E. faecium* strains, respectively. Moreover, only 6 *E. faecalis* and 12 *E. faecium* isolates were resistant to vancomycin, which is comparable to our results (30). In the present study, we found resistance to the first-line treatment, namely aminoglycosides. We also observed resistance to substituting antibiotics, i.e. vancomycin and teicoplanin, though at low levels, especially for vancomycin. Based on evidence, the high transformability of glycopeptides in Enterococci help develop resistance to different antibiotics, an alarming commencement of increased resistance to common antibiotics in Enterococci (31).

In our study, a strong positive correlation was found between resistance to vancomycin and teicoplanin, with the similar rate of resistance. This observation suggests that if one of these two antibiotics is ineffective in the treatment of an Enterococci infection, the other one seems also to be inefficient. Hence, their prescription is not recommended owing to the similarity of the action mechanism of the two antibiotics, which both are from glycopeptides family. These inhibit growth of bacteria by interfering peptidoglycan biosynthesis.

Among 129 Enterococci spp. isolates, 59 (45%) and 21 (20%) *E. faecium* and 27 (20%) and 0.04% *E. faecalis* isolates were positive for the presence of *vanA* and *vanB* genes, respectively. Moreover, 57.1% of VRE *E. faecium* and 57.1% VRE *E. faecalis* isolates were positive for the presence of both *vanA* and *vanB* genes. These genes mediate the synthesis of abnormal precursors in bacterial peptidoglycan, hence reducing vancomycin affinity for binding to the peptidoglycan. The results of our study, the same as others, recognized *vanA* and *vanB* as the most prevalent and predominant types of vancomycin-resistant genes (32). In VRE *E. faecalis* isolates, 57.1% was positive for the presence of *aac (6’)*. In addition, 85 Enterococci spp. and 103 Enterococci spp., including *E. faecium* (79%) and *E. faecium* (58%), isolates carried *aac (6’)* and *aph (2’)*, respectively. In Diab et al.’s (2019) study, the enterococci isolates carried aminoglycoside modifying *aac (6’)-le-aph (2’)-la* gene in 26/39 (66.7%) of Enterococci spp., whereas 32/37 (86.5%) of Enterococci spp. carried *aph (3’)-lla* gene and were observed in both *E. faecalis* and *E. faecium* (33).

**Conclusion**

In sum, we showed a detailed resistance pattern of clinically isolated Enterococci species to common antibiotics. Our results are not merely descriptive; using statistical analysis, we distinguish between resistance patterns that may have occurred due to chance alone and patterns that are unlikely to have occurred by chance. Our findings showed positive and negative correlations between the resistance to common antibiotics in these bacteria. Accordingly, the results confirmed the association between simultaneous resistance to vancomycin and teicoplanin. These results can guide antibiotic prescriptions against Enterococci infections.

**Declarations**

**Ethics approval and consent to participate**

This study was approved by the Ethics Committee of Ahvaz Jundishapur University of Medical Sciences (IR.AJUMS.REC.1396.583) Ahvaz, Iran. This study used data collected from patient records while maintaining patient anonymity. Because this study presented no more than minimal risk of harm to patient subjects, the institutional review board approved a waiver of patient informed consent.

**Consent for publication**

Not applicable.

**Availability of data and materials**
All data generated or analyzed during this study are included in the present published article and its supplementary information file.

**Competing interests**

The authors report no conflicts of interest in this work.

**Funding**

Not applicable.

**Authors' contributions**

The concept and the design of the study were developed E.A. The methodology was designed by A.K. Data collection and the experimental works were carried out by k.A and M.A. The original draft was prepared by A.A. All the authors have read and approved the final manuscript for submission.

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**Tables**

**Table 1**- Patient demographics, antibiotic resistance and genotypic differentiate in Enterococcus spp
| ID | G/AGE | specimen         | 16S rDNA | ARPs       | vancomycin | Genes          |
|----|-------|------------------|----------|------------|------------|----------------|
| 1  | 38-M  | wound            | *E. faecium* | G,E,Ts,CIP,IMI,SAM,TEC,P,T | -          | VANA,aac,aph.INT1 |
| 2  | 21-F  | wound            | *E. faecium* | E,Ts,CIP,SAM,TEC,P,T | R          | VANB,aac,aph.INT1 |
| 3  | 31-M  | wound            | *E. faecium* | E,Ts,CIP,SAM,TEC,P,T | -          | VANA,aac,aph.INT1 |
| 4  | 54-M  | wound            | *E. faecium* | E,Ts,CIP,SAM,TEC,P,T | R          | VANA,aac,aph.INT1 |
| 5  | 13-F  | song             | *E. faecalis* | G,E,Ts,CIP,SAM,TEC,P,T | R          | VANA,aac,aph.INT1 |
| 6  | 36-M  | wound            | *E. faecium* | G,E,Ts,CIP,SAM,TEC,P,T | -          | VANA,aac,aph.INT1 |
| 7  | 26-F  | wound            | *E. faecium* | G,E,Ts,CIP,SAM,TEC,P,T | R          | VANA,aac,aph.INT1 |
| 8  | 30-F  | secretory-ear    | *E. faecium* | E,Ts,CIP,SAM,TEC,P,T | -          | VANA,aac,aph.INT1 |
| 9  | 70-F  | wound            | *E. faecalis* | G,Ts,CIP,SAM,TEC,P,T | -          | VANA,aac,aph.INT1 |
| 10 | 24-M  | Urine            | *E. faecium* | E,Ts,CIP,SAM,TEC,P,T | R          | VANA,aac,aph.INT1 |
| 11 | 47-M  | Song             | *E. faecium* | E,Ts,CIP,SAM,TEC,P,T | -          | VANA,aac,aph.INT1 |
| 12 | 35-M  | wound            | *E. faecium* | G,Ts,CIP,SAM,TEC,P,T | R          | VANA,aac,aph.INT1 |
| 13 | 12-M  | wound            | *E. faecium* | E,Ts,CIP,SAM,TEC,P,T | -          | VANA,aac,aph.INT1 |
| 14 | 38-M  | Urine            | *E. faecium* | E,Ts,CIP,SAM,TEC,P,T | -          | VANA,aac,aph.INT1 |
| 15 | 32-M  | wound            | *E. faecium* | G,Ts,CIP,SAM,TEC,P,T | R          | VANA,aac,aph.INT1 |
| 16 | 36-F  | wound            | *E. faecium* | E,Ts,CIP,SAM,TEC,P,T | -          | VANA,aac,aph.INT1 |
| 17 | 65-M  | wound            | *E. faecium* | E,Ts,CIP,TEC,P,T | R          | VANB,aac,aph.INT1 |
| 18 | 52-F  | secretory-nose   | *E. faecium* | G,Ts,CIP,SAM,TEC,P,T | -          | VANA,aac,aph.INT1 |
| 19 | 49-F  | wound            | *E. faecium* | E,Ts,CIP,TEC,P,T | R          | VANA,aac,aph.INT1 |
| 20 | 66-M  | Urine            | *E. faecium* | E,Ts,CIP,TEC,P,T | -          | VANB,aac,aph.INT1 |
| 21 | 57-F  | Urine            | *E. faecium* | G,Ts,CIP,SAM,TEC,P,T | R          | VANA,aac,aph.INT1 |
| 22 | 10-M  | Urine            | *E. faecium* | E,Ts,CIP,TEC,P,T | -          | VANA,aac,aph.INT1 |
| 23 | 14-F  | Urine            | *E. faecalis* | E,Ts,CIP,PT | -          | VANB,aac,aph.INT1 |
| 24 | 22-F  | Urine            | *E. faecium* | G,Ts,CIP,PT | R          | VANA,aac,aph.INT1 |
| 25 | 39-M  | Urine            | *E. faecium* | E,Ts,CIP,PT | -          | VANB,aac,aph.INT1 |
| 26 | 39-F  | Urine            | *E. faecium* | G,Ts,CIP,PT | R          | VANA,aac,aph.INT1 |
| 27 | 64-M  | Urine            | *E. faecalis* | E,Ts,CIP,PT | -          | VANA,aac,aph.INT1 |
| 28 | 53-M  | Urine            | *E. faecium* | G,Ts,CIP,PT | -          | VANB,aac,aph.INT1 |
| 29 | 43-M  | Urine            | *E. faecalis* | E,Ts,CIP,PT | R          | VANB,aac,aph.INT1 |
| 30 | 23-M  | Urine            | *E. faecium* | G,Ts,CIP,PT | -          | VANA,aac,aph.INT1 |
| 31 | 25-F  | Urine            | *E. faecalis* | TS,CIP,PT | R          | VANA,aac,aph.INT1 |
|   |   |   |   |   |   |   |   |   |   |   |
|---|---|---|---|---|---|---|---|---|---|---|
| 32 | 32-F | Urine | *E. faecium* | E,Ts,CIP,P,T | - | VANA,aac,aph |
|   |   | Urine | *E. faecalis* | G,Ts,CIP,P,T | - | Aac,aph |
| 33 | 30-M | Urine | *E. faecalis* | E,Ts,CIP,P,T | R | VANB,aac,aph |
| 34 | 21-M | Urine | *E. faecium* | G,Ts,CIP,P,T | - | VANA,aac,aph |
| 35 | 61-M | Urine | *E. faecalis* | E,Ts,CIP,P,T | R | VANA,aac,aph |
| 36 | 39-F | Urine | *E. faecium* | E,Ts,CIP,P,T | - | VANA,aac,aph |
| 37 | 15-M | Urine | *E. faecalis* | G,Ts,CIP,P,T | R | VANB,aac,aph |
| 38 | 46-F | Urine | *E. faecium* | E,Ts,CIP,P,T | - | VANA,aac,aph |
| 39 | 40-F | Urine | *E. faecalis* | G,Ts,CIP,P,T | R | VANA,aac,aph |
| 40 | 35-M | Urine | *E. faecium* | E,Ts,CIP,P,T | R | VANB,aac,aph |
| 41 | 42-F | Urine | *E. faecalis* | E,Ts,CIP,P,T | - | VANA,aac,aph |
| 42 | 19-F | Urine | *E. faecium* | TS,CIP,P,T | R | VANA,aac,aph |
| 43 | 29-F | Urine | *E. faecalis* | E,Ts,CIP,P,T | - | VANA,aac,aph |
| 44 | 17-F | Urine | *E. faecium* | E,Ts,CIP,P,T | R | VANA,aac,aph |
| 45 | 23-M | wound | *E. faecalis* | G,E,Ts,CIP,P,T | - | VANB,aac,aph |
| 46 | 26-M | wound | *E. faecium* | E,Ts,CIP,P,T | R | VANB,aac,aph |
| 47 | 45-F | wound | *E. faecalis* | G,E,Ts,P,T | R | VANA,aac,aph |
| 48 | 60-M | wound | *E. faecalis* | E,Ts,P,T | - | VANA,aac,aph |
| 49 | 50-F | wound | *E. faecalis* | E,Ts,P,T | R | VANB,aac,aph |
| 50 | 42-M | wound | *E. faecalis* | G,E,Ts,P,T | - | VANA,aac,aph |
| 51 | 22-M | wound | *E. faecalis* | G,E,Ts,P,T | R | VANB,aac,aph |
| 52 | 16-M | Urine | *E. faecalis* | E,Ts,P,T | - | VANA,aac,aph |
| 53 | 55-M | Urine | *E. faecalis* | G,Ts,P,T | R | VANA,aac,aph |
| 54 | 76-M | Urine | *E. faecium* | E,Ts,P,T | - | VANB,aac,aph |
| 55 | 43-F | Urine | *E. faecalis* | E,Ts,P,T | - | VANA,aac,aph |
| 56 | 12-M | Urine | *E. faecalis* | E,Ts,P,T | - | VANB,aac,aph |
| 57 | 34-F | Urine | *E. faecalis* | G,Ts,P,T,RP | R | VANA,aac,aph |
| 58 | 35-M | Urine | *E. faecium* | E,Ts,P,T,RP | - | VANA,aac,aph |
| 59 | 45-M | Urine | *E. faecalis* | E,Ts,T,RP | - | VANA,aac,aph |
| 60 | 32-F | Urine | *E. faecium* | G,E,Ts,T,RP | - | VANB,aac,aph |
| 61 | 32-F | Urine | *E. faecalis* | E,Ts,T,RP | - | VANA,aac,aph |
| 62 | 13-M | Urine | *E. faecium* | E,Ts,T,RP | R | VANA,aac,aph |
| 63 | 44-M | Urine | *E. faecalis* | G,E,Ts,T | - | VANA,aac,aph |
|   |   |   |   |   |   |   |
|---|---|---|---|---|---|---|
|64 | 21-F | Urine | *E. faecium* | E,Ts,T | - | VANB,aac,aph |
|65 | 65-F | Urine | *E. faecalis* | G,Ts,T | - | VANA,aac,aph |
|66 | 44-F | Urine | *E. faecium* | E,Ts,T | - | VANA,aac,aph |
|67 | 31-M | Urine | *E. faecium* | G,Ts,T,RP | - | VANB,aac,aph |
|68 | 54-M | Urine | *E. faecalis* | E,Ts,T,RP | - | VANA,aac,aph |
|69 | 33-F | Urine | *E. faecium* | G,Ts,T,RP | - | VANB,aac,aph |
|70 | 53-F | Urine | *E. faecium* | E,Ts,RP | - | VANA,aac,aph |
|71 | 40-F | wound | *E. faecium* | G,Ts,RP | - | VANA,aac,aph |
|72 | 45-F | wound | *E. faecium* | E,Ts,RP | - | VANA,aac,aph |
|73 | 23-F | wound | *E. faecium* | G,Ts | - | VANA,aac,aph |
|74 | 30-M | wound | *E. faecium* | E,Ts,RP | - | VANA,aac,aph |
|75 | 34-F | wound | *E. faecium* | G,Ts,RP | - | VANA,aac,aph |
|76 | 33-F | wound | *E. faecium* | E,Ts | - | VANA,aac,aph |
|77 | 24-M | wound | *E. faecium* | G,Ts,RP | - | VANA,aac,aph |
|78 | 32-M | wound | *E. faecium* | E,Ts,RP | - | VANA,aac,aph |
|79 | 43-M | wound | *E. faecium* | E,Ts,RP | - | VANA,aac,aph |
|80 | 32-M | wound | *E. faecium* | E,Ts,RP | - | VANA,aac,aph |
|81 | 23-M | wound | *E. faecium* | G,Ts,RP | - | VANA,aac,aph |
|82 | 64-M | wound | *E. faecium* | E,Ts,RP | - | VANA,aac,aph |
|83 | 33-F | wound | *E. faecium* | G,Ts,RP | - | VANA,aac,aph |
|84 | 37-F | wound | *E. faecium* | E,Ts,RP | - | VANA,aac,aph |
|85 | 30-M | wound | *E. faecium* | E,CIP,RP | - | VANA,aac,aph |
|86 | 68-F | wound | *E. faecium* | G,CIP,RP | - | VANA,aph |
|87 | 14-F | wound | *E. faecium* | E,CIP,RP | - | VANA,aph |
|88 | 12-F | wound | *E. faecium* | G,CIP,RP | - | VANA,aph |
|89 | 60-M | wound | *E. faecium* | E,CIP,RP | - | VANA,aph |
|90 | 43-M | wound | *E. faecium* | G,CIP | - | VANA,aph |
|91 | 22-M | wound | *E. faecium* | E,CIP | - | VANA,aph |
|92 | 23-M | wound | *E. faecium* | E,CIP | - | VANA,aph |
|93 | 43-M | wound | *E. faecium* | G,CIP | - | VANA,aph |
|94 | 34-M | wound | *E. faecium* | E,CIP | - | VANA,aph |
|95 | 43-M | wound | *E. faecium* | G,CIP | - | VANA,aph |
| Case | Age | Type  | Pathogen | Antimicrobials | Comments |
|------|-----|-------|-----------|----------------|----------|
| 96   | 22-M| wound | E. faecium | E, CIP | - | VANA, aph, INT2 |
| 97   | 33-M| wound | E. faecium | G, CIP | - | VANA, aph, INT2 |
| 98   | 43-M| wound | E. faecium | E, CIP | - | VANA, aph, INT2 |
| 99   | 43-M| sond  | E. faecium | G, CIP | - | VANA, aph, INT2 |
| 100  | 22-M| wound | E. faecium | E, CIP | - | VANA, aph, INT2 |
| 101  | 13-F| wound | E. faecium | G, CIP | - | VANA, aph, INT2 |
| 102  | 53-M| wound | E. faecium | E, CIP | - | VANA, aph, INT2 |
| 103  | 34-M| wound | E. faecium | G, CIP | - | VANA, aph, INT2 |
| 104  | 22-F| wound | E. faecium | E, CIP | - | VANA, INT2 |
| 105  | 44-M| sond  | E. faecium | G, E, CIP | - | VANA, INT2 |
| 106  | 33-F| wound | E. faecium | G, E, CIP | - | VANA |
| 107  | 33-F| Urine | E. faecium | G, E, CIP, IMI | - | VANA |
| 108  | 34-F| Urine | E. faecium | G, E, CIP, IMI | - | VANA |
| 109  | 23-F| Urine | E. faecium | G, E, CIP, IMI | - | VANA |
| 110  | 43-M| Urine | E. faecium | G, E, CIP, IMI | - | VANA |
| 111  | 53-F| Urine | E. faecium | G, E, CIP, IMI | - | VANA |
| 112  | 32-M| Urine | E. faecium | G, E, IMI | - | VANA |
| 113  | 12-M| Urine | E. faecalis | IMI | - | VANA |
| 114  | 17-F| Urine | E. faecalis | IMI | - | VANA |
| 115  | 64-F| Urine | E. faecalis | G, E, IMI | - | VANA |
| 116  | 43-M| Urine | E. faecalis | G, E, IMI | - | VANA |
| 117  | 56-M| Urine | E. faecalis | G, E, IMI | - | VANA |
| 118  | 54-M| Urine | E. faecalis | G, E, IMI | - | VANA |
| 119  | 65-F| Urine | E. faecalis | G, E, IMI | - | VANA |
| 120  | 25-M| Urine | E. faecalis | G, E, IMI, SAM | - | VANA |
| 121  | 34-F| Urine | E. faecalis | G, E, IMI, SAM | - | VANA |
| 122  | 64-F| Urine | E. faecalis | G, E, IMI, SAM | - | VANA |
| 123  | 35-F| Urine | E. faecalis | IMI, SAM | - | VANA |
| 124  | 63-M| Urine | E. faecalis | IMI, SAM | - | VANB |
| 125  | 24-M| Urine | E. faecalis | IMI, SAM | - | VANB |
| 126  | 56-F| Urine | E. faecalis | IMI, SAM | - | VANB |
| 127  | 36-F| Urine | E. faecium | IMI, SAM, TEC | - | VANB |
| 128  | 35-F| Urine | E. faecium | IMI, SAM, TEC | - | VANB |
• Penicillin (P), Imipenem (IMI), Gentamicin (G), Ciprofloxacin (CIP), Erythromycin (E), ampicillin-sulbactam (SAM), Teicoplanin (TEC)

**Table 2**- shows the total distribution of *E. faecalis* and *E. faecium* in various types of clinical specimens in hospital

| Clinical Specimens | *E. faecalis* | *E. faecalis* | *E. faecium* | *E. faecium* |
|--------------------|--------------|--------------|--------------|--------------|
|                    | VRE          | Non-VRE      | VRE          | Non-VRE      |
| Wound              | 3            | 5            | 8            | 40           |
| Urine              | 9            | 30           | 8            | 23           |
| * sond*            | 1            | -            | -            | 2            |
| Secretary nose     | -            | -            | -            | 1            |
| Secretary ear      | -            | -            | -            |              |

**Table 3** - Results of antimicrobial resistance tests for *E. faecalis* by disk diffusion method by

| Antimicrobial category | Antimicrobial agent         | resistant |
|------------------------|----------------------------|-----------|
| PENICILLINS            | Penicillin                 | 23(17%)   |
| LIPOGLYCOPEPTIDES      | Teicoplanin                | 5(0.03%)  |
| CARBAPENEMS            | Imipenem                   | 15(11%)   |
| AMINOGLYCOSIDES        | Gentamycin                 | 23(40%)   |
| FLUOROQUINOLONES       | Ciprofloxacin              | 16(12%)   |
| MACROLIDES             | Erythromycin               | 31(24%)   |
| TETRACYCLINES          | Tetracycline               | 28(21%)   |
| ANSAMYCINS             | Rifampin                   | 6(0.04%)  |
| FOLAT PATWAYANTAGONISM | Trimethoprim/Sulfamethoxazole | 25(19%)  |
| B-LACTAM COMBINATION AGENT | ampicillin-sulbactam     | 10(7%)    |
| GLYCOPEPTIDES          | Vancomycin                 | 12(9%)    |

**Table 4** - Results of antimicrobial resistance tests for *E. faecium* by disk diffusion method by
| Antimicrobial category       | Antimicrobial agent          | resistant |
|-----------------------------|------------------------------|-----------|
| PENICILLINS                 | Penicillin                   | 33(25%)   |
| LIPOGLYCOPEPTIDES           | Teicoplanin                  | 22(17%)   |
| CARBAPENEMS                 | Imipenem                     | 8(0.06%)  |
| AMINOGLYCOSIDES             | Gentamycin                   | 37(38%)   |
| FLUOROQUINOLONES            | Ciprofloxacin                | 55(42%)   |
| MACROLIDES                  | Erythromycin                 | 52(40%)   |
| TETRACYCLINES               | Tetracycline                 | 38(29%)   |
| ANSAMYCINS                  | Rifampin                     | 23(17%)   |
| FOLAT PATWAYANTAGONISM      | Trimethoprim/Sulfamethoxazole| 50(38%)   |
| B-LACTAM COMBINATION AGENT | ampicillin-sulbactam         | 18(7%)    |
| GLYCOPEPTIDES               | Vancomycin                   | 16(12%)   |

Table 5- Distribution of in of class 1 and 2 and 3 integrons in vancomycin and aminoglycoside resistance genes in Enterococcus spp

| VA  | VB  | AAC | APH |
|-----|-----|-----|-----|
| R   | S   | P   | R   | S   | P   | R   | S   | P   | R   | S   | P   |
| *0.008 | 0.803 | 0.053 | *0.038 |
| INT1 |     |     |     |     |     |     |     |     |     |     |     |
| R   | 27  | 4   | 9.3 | 7   | 24  | 23.5 | 25  | 6   | 13.6 | 29  | 2   | 7.7 |
| S   | 59  | 39  | 90.7 | 20  | 78  | 76.5 | 60  | 38  | 86.4 | 74  | 24  | 92.3 |

INT2 | 0.480 | 0.684 | *0.028 | 0.686 |
| R   | 5    | 4    | 9.3   | 1    | 8    | 7.8   | 9    | 0    | 0.0  | 8    | 1    | 3.8 |
| S   | 81   | 39   | 90.7  | 26   | 94   | 92.2  | 76   | 44   | 100.0 | 95   | 25   | 96.2 |

Values are expressed as number (%).
P values based on Fisher's exact test.
*It is statistically significant

Figures
Figure 1

The prevalence of Enterococcos SPP among clinical specimens