Section: Microbiology

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# A STUDY ON HIGH-LEVEL AMINOGLYCOSIDE RESISTANCE IN CLINICAL ISOLATES OF ENTEROCOCCUS SPECIES: A TERTIARY CARE HOSPITAL-BASED STUDY

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## ABSTRACT

**Background:** To know the antimicrobial susceptibility pattern of enterococcus species isolated from various clinical specimens. To find the prevalence of high-level aminoglycoside resistance among these isolates by disc diffusion method. **Introduction:** Enterococci species are classified as high-priority bacteria concerning antimicrobial resistance. *E. faecalis* and *E. faecium* are the most common species accounting for 95% of the clinical isolates and are known to express intrinsic resistance to low levels of aminoglycosides. Enterococcal species enzymes increase the MIC for aminoglycosides, preventing synergism. Combined resistance is extremely difficult in treating infections caused by Enterococci species. **Materials and Methods:** A cross-sectional study was conducted over a period from January 2021 to December 2022 in Microbiology Laboratory, B.J.GMC, Pune. A total of 102 Enterococci species were included in the present study. Antimicrobial susceptibility including high level aminoglycoside resistance was performed by Kirby-Bauer disc diffusion method as per CLSI M 100-Ed32 2022 guidelines. Data were compared with the Chi-Square and Fishers' exact test. **Result:** Out of 102 Enterococci isolated, 53 (51.96%) were *E. faecium*, 44 (43.14%) were *E. faecalis*, and only five were others. Maximum enterococci were isolated from females patients (54%). Urine being the most common samples from which enterococcal strains were isolated (74.51%) and indoor patient had more enterococcal infection 64.70% than outdoor patients 17.64%. **Conclusion:** Multidrug resistance patterns are seen in enterococcal strains, which emphasizes the need for more rational use of antimicrobials. The rapid rise of antimicrobial resistance among hospital-adapted enterococci has rendered nosocomial infections a leading therapeutic challenge.

## INTRODUCTION

Enterococcus species are indigenous flora of the intestinal tract, oral cavity & genitourinary tract of humans and animals.[1,2] Enterococci are considered as having relatively low virulence but they are known to cause various clinical infections like urinary tract infections, endocarditis, intra-abdominal and pelvic infections, neonatal sepsis, and meningitis.[3,4] In recent times it has emerged as a troublesome nosocomial pathogen causing significant morbidity and mortality and a cause of nosocomial superinfection in patients receiving antimicrobial agent.[5]

Globally the prevalence of multidrug resistance among enterococci was found to be 63%, the resistance being more common in *Enterococcus faecium* as compared to *Enterococcus faecalis*.[6] In India, high-level aminoglycoside resistance (HLAR) has been reported from different centres. Elango Padmasini *et al*. (Chennai, India) have reported a prevalence rate of high-level resistance of 42.7% for Gentamicin, 29.8% for streptomycin, and 21.9% for the combine.[7] Enterococci are considered an important difficult-to-treat pathogen, due to their intrinsic resistance to several antimicrobial agents like cephalosporins, lincosamides, low levels of aminoglycosides, and many β-lactam agents. These resistances are acquired by means of mutations or as a result of the transfer of genes located in plasmids/transposons.[8,9]

All enterococci have intrinsic low-level resistance to aminoglycosides, with minimal inhibitory concentrations (MICs) ranging from 4µmL to as high as 256 µg/mL. These can be overcome by addition of an agent that interferes with cell wall synthesis, such as ampicillin or vancomycin, which can increase the uptake of the aminoglycoside, greatly enhancing the killing of Enterococcus.[10] However as per the literatures high-level aminoglycoside resistance is increasing which is more common in *E. faecium* than in *E. faecalis*.[11] Various studies have shown a higher percentage of high-level gentamicin resistance(HLGR) as compared to high-level streptomycin resistance (HLSR).[12]

The combined resistance of glycopeptides and high-level aminoglycosides is extremely difficult to treat the infections caused by Enterococci.[10,9,13] Due to increased antibiotic resistance among enterococci, the present study was conducted to study the prevalence of high-level aminoglycoside resistance particularly to gentamicin and streptomycin among the clinical isolates of Enterococci in the tertiary care center.

## MATERIAL AND METHODS

A prospective laboratory based cross-sectional study was conducted in department of microbiology, tertiary care hospital, Pune over a period of two year from January 2021 to December 2022. Ethical clearance from the institutional ethical committee was obtained. Various clinical specimens except specimens like sputum, throat swab, and stool as enterococci are known to be commensals in these specimens were processed as per standard microbiological techniques for isolation and identification of Enterococci.[14,15,16] The antimicrobial sensitivity was done by standard Kirby-Bauer disc diffusion method. All the antibiotic discs were procured commercially from Hi-Media Laboratories Pvt . Ltd, Mumbai (Table 1&2). The diameter of the zone of inhibition was measured and interpreted according to CLSI guidelines 2022. *Staphylococcus aureus* ATCC 25923 was used as a control strain.

**Table 1: The antibiotic discs used for ABST**

| Antimicrobial agent | Disc conc. (µg/disc) | Zone of inhibition (mm) | | |
|---|---|---|---|---|
| | | Sensitive | Intermediate | Resistant |
| Penicillin | 10 units | ≥ 15 | - | ≤ 14 |
| Ampicillin | 10 µg | ≥ 17 | - | ≤ 16 |
| Vancomycin | 30 µg | ≥ 17 | 15-16 | ≤ 14 |
| Teicoplanin | 30 µg | ≥ 14 | 11-13 | ≤ 10 |
| Erythromycin | 15 µg | ≥ 23 | 14-22 | ≤ 13 |
| Tetracycline | 30 µg | ≥ 19 | 15-18 | ≤ 14 |
| Ciprofloxacin | 5 µg | ≥ 21 | 16-20 | ≤ 15 |
| Levofloxacin | 5 µg | ≥ 17 | 14-16 | ≤ 13 |
| Norfloxacin | 10 µg | ≥ 17 | 13- 16 | ≤ 12 |
| Nitrofurantoin | 300 µg | ≥ 17 | 15-16 | ≤ 14 |
| Linezolid | 30 µg | ≥ 23 | 21-22 | ≤ 20 |

**Detection of high-level aminoglycoside resistance(HLAR)[17,18]:**

HLAR isolates were identified by the Kirby-Bauer disc diffusion method using the High Level Gentamycin (HLG) disc (120 µg) and High Level Streptomycin (HLS) disc (300 µg). A 0.5 McFarland standard suspension of the isolate was prepared and lawn culture was done on Mueller Hinton Agar. Discs were placed on a medium with the help of sterile forceps. Plates were incubated at $37^0$C for 16-18 hrs. Positive and negative controls were put in simultaneously.

**Table 2: Interpretation of HLAR resistance**

| Antimicrobial agent | Disc conc. (µg/disc) | Zone of inhibition (mm) | | |
|---|---|---|---|---|
| | | Sensitive | Intermediate | Resistant |
| High level Gentamicin (HLG) | 120 µg | ≥ 10 | 7-9 | 6 |
| High-level Streptomycin (HLS) | 300 µg | ≥ 10 | 7-9 | 6 |

**Susceptible control strain:** *E. faecalis* ATCC 29212
**Resistant control strain:** *E. faecalis* ATCC 51299
**Statistical Analysis:** SPSS was used for statistical analysis. Differences in the prevalence of antibiotic resistance between different groups of Enterococcus were assessed by the Chi-square test and Fisher's exact test; results with p<0.05 were considered significant.

## RESULTS

During the study period, a total of 102 isolates of Enterococcus species were obtained from various clinical specimens.

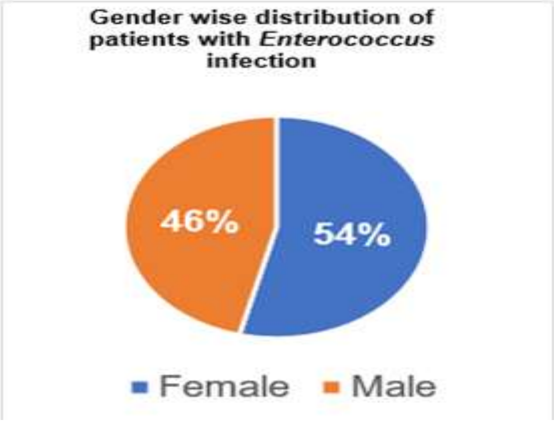


**Figure 1: Gender-wise distribution of patients with *Enterococcus* infection (N=102)**

**Table 3: Age-wise distribution of patients with Enterococcus infection**

| Age group (In years) | No. of cases (%) (N=102) | | |
|---|---|---|---|
| | **Female** | **Male** | **Total** |
| ≤ 10yr | 6(5.88) | 7(6.86) | 13(12.74) |
| 11-20yr | 3(2.94) | 2(1.96) | 5(4.90) |
| 21-30yr | 17(16.67) | 3(2.94) | 20(19.61) |
| 31-40yr | 5(4.90) | 15(14.71) | 20(19.61) |
| 41-50yr | 5(4.90) | 8(7.84) | 13(12.74) |
| 51-60yr | 5(4.90) | 5(4.90) | 10(9.80) |
| ≥ 61yr | 14(13.73) | 7(6.86) | 21(20.59) |
| **Total** | **55(54)** | **47(46)** | **102(100)** |

Figures in parentheses show the percentages

**Table4: Distribution of indoor and outdoor patients with Enterococcus infection (N=102)**

| Type | No. of cases (%) | | |
|---|---|---|---|
| | **Male** | **Female** | **Total** |
| ICU (adult & neonate) | 9(8.82) | 9(8.82) | 18(17.64) |
| Indoor patients | 31(30.39) | 35(34.31) | 66(64.70) |
| Outdoor patients | 7(6.86) | 11(10.78) | 18(17.64) |
| **Total** | **47(46)** | **55(54)** | **102(100)** |

**Table 5: Sample-wise distribution of Enterococcus isolates (N=102)**

| Type of specimen | Female (%) | Male (%) | Total (%) |
|---|---|---|---|
| Blood | 4 (3.92) | 3 (2.94) | 7(6.86) |
| Cerebrospinal fluids | 1(0.98) | 0 | 1(0.98) |
| Pleural fluids | 2(1.96) | 0 | 2(1.96) |
| Pus | 11 (10.78) | 5 (4.90) | 16(15.68) |
| Urine | 37 (36.27) | 39 (38.23) | 76(74.50) |
| **Total** | **55 (54)** | **47 (46)** | **102(100)** |

**Table 6: Species distribution of Enterococcus isolates (N=102)**

| Species | Total No. | Percentage (%) |
|---|---|---|
| *E. faecium* | 53 | 51.96 |
| *E. faecalis* | 44 | 43.14 |
| *E. avium* | 3 | 2.94 |
| *E. durans* | 1 | 0.98 |
| *E. dispar* | 1 | 0.98 |
| **Total** | **102** | **100** |

**Table 7: Sample-wise distribution of various Enterococcus species**

| Enterococcus spp. | Specimen-wise distribution (N=102) | | | | | |
|---|---|---|---|---|---|---|
| | **Blood (n=7)** | **Pleural fluids (n=2)** | **Pus (n=16)** | **CSF (n=1)** | **Urine (n=76)** | **Total** |
| *E. avium* | 0 | 0 | 1 | 0 | 2 | 3 |
| *E. dispar* | 0 | 0 | 1 | 0 | 0 | 1 |
| *E. faecalis* | 6 | 1 | 4 | 0 | 33 | 44 |
| *E. faecium* | 1 | 1 | 10 | 1 | 40 | 53 |
| *E. durans* | 0 | 0 | 0 | 0 | 1 | 1 |
| **Total** | **7** | **2** | **16** | **1** | **76** | **102** |


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**Table 8: Antibiogram of Enterococcus isolates by Kirby-Bauer Disc Diffusion Method:**

| Antibiotics | Enterococcus isolates (N=102) | | |
|---|---|---|---|
| | Resistant (%) | Intermediate Sensitive (%) | Sensitive (%) |
| Penicillin G | 72(70.59) | 0 | 30(29.41) |
| Ampicillin | 70(68.63) | 0 | 32(31.37) |
| High level gentamicin | 50(49.02) | 9(8.82) | 43(42.16) |
| High-level streptomycin | 17(16.67) | 0 | 85(83.33) |
| Ciprofloxacin* | 71(93.42) | 2(2.63) | 3(3.95) |
| Levofloxacin* | 72(94.74) | 0 | 4(5.26) |
| Norfloxacin* | 59(77.63) | 1(1.32) | 16(21.05) |
| Erythromycin** | 17(65.38) | 1(3.85) | 8(30.77) |
| Nitrofurantoin* | 24(31.58) | 0 | 52(68.42) |
| Linezolid | 10(9.80) | 0 | 92(90.20) |
| Vancomycin | 17(16.67) | 0 | 85(83.33) |
| Teicoplanin | 50(49.02) | 0 | 52(50.98) |
| Tetracycline* | 73(96.05) | 1(1.32) | 2(2.63) |

**Table 9: Comparison of antimicrobial resistance pattern among *E. faecalis* and *E. faecium* isolates by Kirby-Bauer Disc Diffusion method**

| Antibiotic name | Resistance pattern among Enterococcus spp. (N=102) | | | | |
|---|---|---|---|---|---|
| | ***E. faecalis*** **(n=44)** | ***E. faecium*** **(n=53)** | **X2** | **P value** | **S/ NS** |
| Penicillin G | 28(63.64) | 40(75.47) | 1.60 | 0.20 | NS |
| Ampicillin | 27(61.36) | 39(73.58) | 0.95 | 0.32 | NS |
| High level gentamicin | 16(36.36) | 33(62.26) | 6.45 | 0.011 | S |
| High-level streptomycin | 5(11.36) | 12(22.64) | 2.12 | 0.15 | NS |
| Ciprofloxacin* | 28(84.85) | 40(100) | 1.61 | 0.20 | NS |
| Levofloxacin* | 33(100) | 36(90) | 0.59 | 0.44 | NS |
| Norfloxacin* | 25(75.76) | 31(77.5) | 0.03 | 0.87 | NS |
| Erythromycin** | 10(90.91) | 6(46.15) | 2.27 | 0.13 | NS |
| Nitrofurantoin* | 8(24.24) | 15(37.5) | 1.36 | 0.24 | NS |
| Linezolid | 3(6.82) | 7(13.21) | 1.06 | 0.30 | NS |
| Vancomycin | 3(6.82) | 14(26.42) | 6.39 | 0.011 | S |
| Teicoplanin | 17(38.64) | 30(56.60) | 3.11 | 0.07 | NS |
| Tetracycline* | 31(93.94) | 39(97.5) | 0.12 | 0.73 | NS |

The degree of freedom for each Chi-square is 1(df= 1)
S = Significant, NS = non-Significant
*Antibiotics tested for urine isolates only, ** antibiotics were tested for other than urine isolates.

**Table10: Distribution of multidrug resistance among Enterococcus species (N=102)**

| No. of antimicrobial Agents | *E. faecalis* (n=44) | *E. faecium* (n=53) | Other enterococci (n=5) | Total (N=102) |
|---|---|---|---|---|
| 3 | 18 | 9 | 2 | 29 |
| 4 | 9 | 14 | 0 | 23 |
| 5 | 4 | 11 | 0 | 15 |
| 6 | 3 | 11 | 1 | 15 |
| 7 | 0 | 0 | 0 | 0 |
| 8 | 0 | 0 | 0 | 0 |
| **Total** | **34(77.27)** | **47(84.91)** | **3(60)** | **82(80.39)** |

Figures in parentheses show the percentages

- In our study, 82 (80.39%) isolates showed a multidrug resistance pattern.
- Multidrug resistance pattern *E. faecium* (84.91%) was higher than *E. faecalis* (77.27%) and other enterococci species (60%).

## DISCUSSION

In the present study, a total of 102 isolates of Enterococcus were obtained from various clinical samples. In our study, male to female ratio among the patients with Enterococcus infection was 1:1.18(Table 3), similar to Study done by Maureen Edwards(2020),[19] Adhikari RP *et al.* (2018),[20] documented male to female ratio as 1:1.48, 1:1.20 respectively. In this study, isolation of Enterococcus was more in patients with ages more than 60 years (20.59%) followed by 21-30 and 31-40 years (19.61%) and below 10 years (12.74%)( Table 3). Similar observations were reported by Maj Puneet Bhatt *et al.* (2015),[21] with maximum number of isolates from patients with the age more than 60 years (30.5%) followed by the age group 21-30 years (25%).

Enterococcus species are the normal flora in gastrointestinal tract which could be the source of infections like urinary tract infections, surgical site infections. Decreased immunity, prolonged hospitalization and other associated co-morbidities can be relatable cause in the age group >60 years and young female are more prone to having urinary tract infections. This might explain the reason why most of the enterococcal isolates were in both gender from reproductive age group 21-30 and 31-40 years in our study.

On comparison with indoor and outdoor patients in regards to enterococcal infections it was seen that majority of Enterococcus isolates were obtained from patients admitted in various wards (64.70%) as compared to ICUs (17.64%) and OPD patients (17.64%) (Table no.4). These findings were similar to the study done by Ashish Karna *et al*. (2019),[22] reported majority from hospitalized patients (61.5%) as compared to emergency (8.8%) and OPD (29.7%), also study done by Adhikari RP *et al*. (2018)[20] reported maximum number inpatients (61.6%) than outpatients (39.4%), Inanimate objects, ranging from the rectal thermometer to air-fluidized microsphere beds and prolonged hospital stays have led to increased colonization of enterococci among hospitalized patients.[23] Many workers have expressed their view that the duration of hospital stay is directly proportional to the prevalence of the infection since the isolation rate is higher among indoor than outdoor patients. This could be attributed to transmission of infection from patient to patient via the hands of nursing staff, thus frequent hand washing to prevent the spread of organisms should be encouraged.

On analysis of sample-wise distribution of Enterococcus isolates (Table no. 5) it was noticed that the isolation of Enterococcus was more from urine samples (74.50%) followed by pus (15.68%) and blood (6.86%) which is somewhat similar to Maureen Edwards(2020),[19] with urine samples (51%) followed by pus (14%) and blood (4%) and the study done by Maj Puneet Bhatt *et al*. (2015),[21] and Kanthishree B H *et al*. (2014),[24] who obtained 57% and 72.2% of isolates from urine followed by pus 16% and 16.6% respectively. The most common nosocomial infections caused by enterococci are urinary tract infections (associated with instrumentation and antimicrobial administration) followed by intra-abdominal and pelvic infections. They also cause surgical site infections, bacteraemia, endocarditis, neonatal sepsis, and rarely meningitis.[21]

In the present study, the most common species isolated was *E. faecium* (51.96%) followed by *E. faecalis* (43.14%) (Table no.6) similar to the study done by Maureen Edwards(2020),[19] Purohit *et al*. (2017),[25] Wei Jia *et al*. (2014)[26], and Raveendranath *et al*. (2017),[27] who showed isolation of *E. faecium* (54%, 64.8%, 58.7%, and 73%) were more than *E. faecalis* (46%, 32.84%, 33% and 24%) respectively. While in a study done by Chaudhary U *et al*. (2007),[28] Bekhit *et al*. (2012),[29] and N. Gangurde *et al*. (2014),[30] isolation of *E. faecalis* (72.3%, 69.2%, and 60%) was more common than *E. faecium* (17.3%, 11.3% and 32.2%) respectively. The Enterococcus genus includes more than 49 species, although only a few can cause clinical infections in humans. *E. faecalis* and *E. faecium* are the most prevalent species accounting for more than 90% of infections.[22,31]

On Sample-wise distribution of all Enterococcus species(Table no.7) it was found that isolation of *E. faecium* was more common than *E. faecalis* in all specimens except blood specimens. Among 76 isolates from urine, 40 were *E. faecium* and 33 were *E. faecalis* and 3 were other enterococcus species, while in a study done by Ashish Karna *et al*. (2019),[22] out of 56 isolates of urine, 29 were *E. faecalis* and 27 were *E. faecium* and also in a study done by Revati Sharma *et al*. (2013),[32] showed that isolation *E. faecalis* was more as compared to *E. faecium* (out of 51 isolates, 17 *E. faecium* and 29 *E. faecalis*) in urine. Isolation of other Enterococcus species found to be more among urine samples (3) as compared to pus (2) samples, which was similar to a study done by Neha Jha *et al*. (2017),[33] showed that isolation of other Enterococcus species was more among urine (18) samples as compared to pus (2) samples. A study done by Ashish Karna *et al*. (2019),[22] showed that isolation of other Enterococcus species, was more among pus (5) samples as compared to urine samples that did not have a single isolate. Enterococci are the normal resident of gastrointestinal tracts. The proximity of urethra and anus in the perineum might account for the high number of Enterococcus isolates from urine samples. The current study revealed that the majority of the Enterococcus isolates were from urine samples.

Antibiogram of Enterococcus isolates were performed by Kirby Bauer disc diffusion method (Table no.8). In the present study, most of the isolates showed resistance to penicillin (70.59%) almost similar to study done by Shrihari N *et al*. (2011),[3] and Gupta V *et al*. (2007)[34] showed (54.90%) and (61.17%) resistance to penicillin respectively, While in the study done by Damrolien Shan *et al*. (2015)[35] higher penicillin resistance was showed (92.59%). Resistance to ampicillin was 68.63% which is similar to the study done by Amit Singh *et al*. (2013),[36] (66%), while M. Paul *et al*. (2017),[37] reported more ampicillin resistance (78.4%).Lower resistance pattern to ampicillin was seen in a study done by Gupta V *et al*. (2007),[34] and Shah L *et al*. (2012),[38] as 38.88% and 40% respectively. The glycopeptides vancomycin, teicoplanin, and newer derivatives are used to treat serious infections due to resistant Gram-positive bacteria. Glycopeptides inhibit bacterial growth by interfering with peptidoglycan biosynthesis.[39] In our study vancomycin and teicoplanin resistance was found to be 16.67% and 49.02% respectively. In comparison to our study, the study done by Ashish Karna *et al*. (2019),[22] showed almost similar resistance to vancomycin (19%) but lower resistance to teicoplanin (4%). A study done by Amit Singh *et al*. (2013),[36] showed a somewhat lower resistance pattern to vancomycin and teicoplanin at 12% and 8% respectively. The antibiogram showed 49.02 % and 16.67 % of resistance to High-level gentamicin and High-level streptomycin respectively by the Kirby Bauer disc diffusion method. The study conducted by Maj Puneet Bhatt *et al*. (2015),[21] documented 65% and 45% resistance to high-level gentamicin and streptomycin respectively while Shah L *et al*. (2012),[38] showed 53% and 40% high-level gentamicin and streptomycin resistance respectively. However, a study done by Jayavarthini M *et al*. (2015),[40] showed minimum resistance to both high-level gentamicin and high-level streptomycin as 4.76% and 5.56% respectively. All enterococci have intrinsic low-level resistance to aminoglycosides, with minimal inhibitory concentrations (MICs) ranging from 4 µg/ml to as high as 256 µg/ml. Enterococci have acquired aminoglycoside resistance genes that encode various aminoglycoside-modifying enzymes, which result in very high resistance to aminoglycosides (MICs usually ≥ 2000 µg/ml), thereby eliminating the synergistic killing effect.[10]

In urine isolates of enterococcus species resistance to tetracycline was found to be 96.05% while in a study done by Das AK *et al*. (2020),[41] resistance to tetracycline was 77.6%. A study done by Amit Singh *et al*. (2013),[36] and Dineshraj Ravi *et al*. (2016),[42] showed a somewhat lower resistance pattern to tetracycline i.e. 60% and (47%) respectively. In urine isolates of enterococcus species resistance to levofloxacin was 94.74% which is higher as compared to the study done by Maj Puneet Bhatt *et al*. (2015),[21] (51%). Resistance to ciprofloxacin was 93.42%, which is higher compared to the study done by Amit Singh *et al*. (2013),[36] (87%) while study done by M Paul *et al*. (2017),[37] reported minimum resistance to ciprofloxacin (28.4%). However, study done by Das AK *et al*. (2020),[41] showed similar resistance patterns for levofloxacin and ciprofloxacin. In our study,77.63 % of enterococci were resistant to norfloxacin isolated from urine sample, which is almost similar to a study done by Das AK *et al*. (2020),[41] (75.7%) and a study done by Purohit *et al*. (2017),[25] with higher resistance to norfloxacin (92.2%). Study done by Dineshraj Ravi *et al*. (2016),[42] (32%) and Preeti Srivastava *et al*. (2013),[43] (23%) showed lower resistance to norfloxacin as compared to our study. In urine isolates of enterococcus species resistance to nitrofurantoin was 31.58 % almost similar to a study done by Das AK *et al*. (2020),[41] (32%) and the study done by Gupta V *et al*. (2007),[34] and M Paul *et al*. (2017),[37] who showed 15.90% and 16.4% lower resistance to nitrofurantoin respectively, while Dineshraj Ravi *et al*. (2016)[42] showed only 4 % resistance to nitrofurantoin. The overall higher resistance pattern in our study may be attributed to the fact that the study was conducted at a tertiary care multispecialty centre with more patients coming from the periphery and small nursing homes, where injudicious use of antibiotics and inadequate infection control policies are prevalent.

On comparison of antimicrobial resistance pattern among *E. faecalis* and *E. faecium* isolates by Kirby-Bauer Disc Diffusion method (Table no.9) it was observed that the isolates possess an intrinsically relative resistance to penicillin and ampicillin. furthermore, *E faecium* is less susceptible to β-lactam agents than *E. faecalis* because their penicillin-binding proteins (PBPs) have lower affinity for these antibiotics and some strains have plasmid-encoded β-lactamase. In our study, resistance to vancomycin and high-level gentamicin was higher among isolates of *E. faecium* as compared to *E. faecalis* and was found to be statistically significant. Resistance was higher to all other antibiotics among isolates of *E. faecium* as compared to *E. faecalis* but statistically non-significant except in isolates other than urine showed erythromycin resistance was higher in *E. faecalis* which was statistically non-significant. Similarly, in a study by Jayvarthinni Manavalan *et al*. (2015),[40] and Wei Jia *et al*. (2014),[26] isolates of *E. faecium* showed a higher resistance pattern than *E. faecalis*. While the study done by Rana D *et al*. (2020),[44] isolates of *E. faecalis* showed a higher resistance pattern than *E. faecium*.

In the present study distribution of multidrug resistance among Enterococcus species was analysed(Table no.10). Multidrug resistance (MDR) is defined as resistance to three or more than three antimicrobial agents. Irregular administration of antibiotics targeting sensitive strains promotes the emergence of resistant strains, especially MDR strains with the ability to colonize the gut lumen of patients, which leads to an increase in the direct and indirect transfer of the genetic material of resistant strains.[45] In our study multidrug resistance was 80.39% which is similar to the study done by Abdulhakim Abamecha *et al*. (2015),[46] who showed MDR as 89.5% while the MDR isolation rate was 35.2% in a study done by Surbhi Mathur *et al*. (2016),[47] and the study done by Rana D *et al*. (2020),[44] the MDR isolation rate was 51%. Distribution of multidrug resistance was somewhat similar among *E. faecalis* (77.27%), *E. faecium* (84.91%), and other enterococci (60%) which was similar, to a study done by Neha Jha *et al*. (2017)[33] and Maj Puneet Bhatt *et al*. (2015),[21] showed that isolation rate of MDR was more in *E. faecium* (58.82%, 72%) than in *E. faecalis* (28.35%, 45%) respectively. However, the study done by Rana D *et al*. (2020),[44] showed that the isolation rate of MDR was more in *E. faecalis* (57.1%) than in *E. faecium* (52.3%).

The present study reveals the problem of multidrug-resistant Enterococcus isolates and the emergence of VRE and HLAR. Thus, we suggest the rational use of antibiotics in healthcare settings and more surveillance studies to monitor changes in enterococcal resistance patterns. According to CLSI guidelines, all intermediate sensitive or inconclusive strains by disc diffusion method should be tested by screen agar and micro broth dilution method to confirm high-level aminoglycosides resistance in enterococci.[17,18,48] Multiple drug-resistant enterococci pose a grave concern in hospital setting, leading to therapeutic failure in patients. As a significant number of clinical enterococci were positive for both HLGR and HLSR, there is a need to treat infections with high-level aminoglycoside resistance in optimal dosage and duration.it is therefore, imperative for laboratories to endow with precise antimicrobial resistance phenotypes for enterococci so that efficient remedies and measures for infection control can be initiated.

## CONCLUSION

The emergence of high-level resistance to aminoglycosides, β-lactam antibiotics, and vancomycin with the association of multidrug resistance has led to the failure of synergistic effects of combination therapy for enterococcal infections. This would necessitate routine testing of the isolates for high level aminoglycoside resistance. Steps should be taken to regularly screen the enterococcal infections effectively, to limit the spread of multidrug resistant enterococcal infections. Precise identification of enterococci to species level enables us, to assess the species-specific antimicrobial resistance characteristics, apart from knowing the epidemiological pattern and their clinical significance in human infections. A high rate of resistance to most antimicrobials agents is observed in this study and the emergence of HLAR strains has further worsened this situation. Thus, we suggest promoting more rational use of antibiotics in healthcare settings, and more surveillance studies to monitor changes in enterococcal resistance patterns. A good antibiotic policy should be laid down between the clinician and microbiologist in all tertiary care hospitals and a strict antibiotic regimen should be applied by clinicians.

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**Ethical committee approval:**

**Study Location:** A Laboratory Microbiology Department at B.J.GMC. and Sassoon Hospital, PUNE. (A Tertiary care hospital) Study time period: 2 years (01 Jan 2021- 31 Dec 2022)

- ✓ Approval of research from intuitional ethic committee (BORS )on dated 22.01.2021
- ✓ Title and synopsis approval from MUHS University on dated 09.03.2021
- ✓ Dissertation (research) approved by university on dated 23.06.2023
- ✓ Research data presented as oral paper in 27th Maharashtra state conference (state chapter of Indian Association of Medical Microbiologists dated 8th to10th September 2023)

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