



## Increasing Trend of Multi-Drug Resistance Enterococci

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### ABSTRACT:

**Background:** The emergence of Enterococci with multi-drug-resistance has consistently raised concerns among healthcare professionals. They commonly cause urinary tract infection along with blood stream infection, endocarditis, and other nosocomial infections. Enterococcus infections have usually been treated with aminoglycosides and cell wall-active medications; however, as multidrug-resistant strains of the infection have proliferated, the number of effective treatments has dropped. This study aims to ascertain the enterococci's occurrence and vulnerability pattern in clinical samples.

**Material and Methods:** The Facklam and Collins technique was used to identify 312 Enterococcus that were isolated from different clinical samples. The isolates were subjected to ascertain Van A and Van B resistance with the help of modified Kirby Bauer disc diffusion method

**Results:** A total of 312 *Enterococcus* species were obtained from numerous medical samples in which 162 were *E. faecalis*, 48 of *E. avium*, and 102 were *E. faecium*. Resistance to tetracycline, penicillin, ampicillin, ciprofloxacin, and high-level gentamycin were observed. Most of the vancomycin resistant enterococci were observed in urine sample.

**Conclusion:** A high rate of resistance to tetracycline, ciprofloxacin, high-level gentamycin, and erythromycin was found and the situation was further worsened by the emergence of Vancomycin Resistant Enterococci. We, therefore, suggest that, there should be a strict hospital infection policy and practices along with regular screening for enterococcus. The research emphasizes the significant therapeutic challenge posed by the rise in multidrug-resistant enterococci and their rising prevalence.

### 1. Introduction

Infectious disease remains a global health challenge, causing substantial morbidity and mortality. The spread of resistance remains one of the key factors which contributes to nosocomial infections. Enterococci are gram-positive, facultatively anaerobic organism which is found in the alimentary canals of humans. Classified earlier as Group D streptococci is now into a separate genus.<sup>1</sup> They thrive between 10°C - 45°C, withstanding 60°C for 30 minutes, grows in 6.5% NaCl, and at pH 9.6.<sup>2</sup> In recent years, Enterococcus species emerged as one of the most prevalent nosocomial pathogens<sup>4</sup>. They inhabit the gastrointestinal and biliary tracts normally, and are

also found in smaller quantities in the male urethra and female vagina. However, they could turn into pathogens if they colonize areas where they are not often present.<sup>2</sup>

Antibiotic-resistant bacteria are thought to be the cause of approximately 50% of nosocomial infections<sup>3</sup>. Considered to be of low virulence; these are known to cause various clinical infections like urinary tract infections (UTI), endocarditis, intra-abdominal and pelvic infections and nosocomial infections.<sup>5,6</sup> Enterococci currently rank second in the hospital-associated bacteraemia and play significant role in surgical site infections (SSI) and nosocomial UTI.



Additionally, 5–20% of endocarditis acquired in the community is caused by enterococci<sup>7</sup>.

Gram-positive bacteria may develop and spread a wide spectrum of resistance features, making enterococci indicator organisms for the detection of antimicrobial resistance in bacteria<sup>8</sup>. Treatment for enterococcal infections is severely hampered by enterococci's innate resistance to antibiotics as well as their propensity to acquire and spread genetically mobile antibiotic resistance components. Enterococcal infections were treated with cell wall active agents plus an aminoglycoside; however, certain strains of the bacteria have developed resistance to  $\beta$ -lactam antibiotics, vancomycin, and high-level aminoglycoside resistance (HLAR), which has prevented combination therapy from having its intended synergistic effects.<sup>9</sup> This suggests that there is a need for ongoing bacterial surveillance. There is a limited study on drug resistance Enterococci in India. This particular research was undertaken to recognize the drug resistance pattern and the occurrence of Enterococci in a tertiary care hospital found in Southern India.

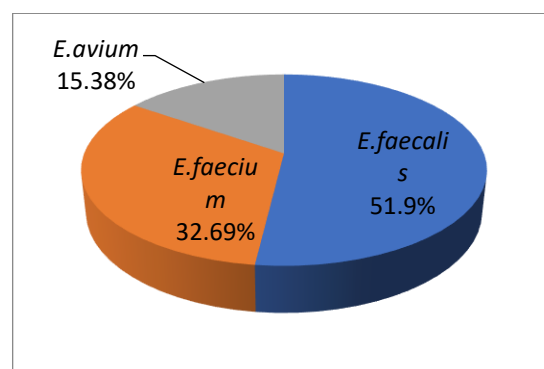
## 2. Methodology

The current work was carried out over two-years, in the department of Microbiology at JSS Medical College after obtaining institutional ethical clearance. A total of 312 strains from various clinical samples were collected from various clinical samples viz. Urine, Pus, Bile, Vaginal Swab etc. The strains were identified and speciation was done according to standard laboratory procedures as per the scheme of Facklam and Collins<sup>13</sup> and antibiotic susceptibility was determined by Kirby Bauer disc diffusion method. Various antibiotics included were Penicillin, High level Gentamicin, Ciprofloxacin, Erythromycin, Linezolid, Teicoplanin, Vancomycin, Tetracyclin and Nitrofurantoin. *E.faecalis* ATCC 29212 was used as control.

## Results

Among 312 strains, 162 were of *E.faecalis*, 102 were found to be of *E.faecium*, and 48 of *E.avium* as shown in Figure 1. In this current study, *E.faecalis* predominates with 51.9%. The various isolates of Enterococci obtained from different samples are detailed in Table 1. In urine, sputum, and abscess samples, *E.faecalis* was found in higher numbers followed by *E.faecium*. In the vaginal samples, 5.12% of *E.avium* was present followed by 2.56% *E.faecium*. There were no specimens of *E.avium* present in the throat, abscess, bile, and pus samples. In bile and throat samples, there were an equal number of *E.faecium* and *E.faecalis* present in both sample respectively. The antibiotic sensitivity test of enterococcus is mentioned in Table 2. It has been found that both *E.avium* and *E.faecalis* were more resistant to tetracycline whereas *E.faecium* were more resistant to erythromycin. In the case of Vancomycin, it was found that *E.faecalis* was more resistant to Vancomycin whereas only 8 isolates of *E.faecium* and 4 isolates of *E.avium* were shield from Vancomycin as shown in Table 3. Vancomycin-resistant enterococcus with different studies have been compared in Table 4.

**Figure 1:** Percentage (%); Enterococci species that were isolated throughout our research



**Table 1: Frequency of isolates derived from different clinical specimens**

Sample	SPECIES NAME		
	<i>E.faecalis</i>	<i>E.faecium</i>	<i>E.avium</i>
Urine	118	58	28
Vaginal Swab	6	8	16
Throat Swab	10	10	0
Sputum	12	6	4
Abscess	8	4	0



Bile	6	6	0
Pus	2	10	0
<b>Total</b>	162	102	48

**Table 2: Enterococci's patterns of antimicrobial resistance as determined by the Kirby Bauer disc diffusion method**

ANTIBIOTIC	SPECIES NAME		
	<i>E.faecalis</i> (n = 162)	<i>E.faecium</i> (n = 102)	<i>E.avium</i> (n = 48)
Ciprofloxacin	126 (77%)	77 (75.4%)	28 (58%)
Erythromycin	110 (I = 16) 67%	81 (I = 4) 79.4%	28 (I = 6) 58%
High level Gentamicin	100 (61%)	54 (52%)	32 (66%)
Linezolid	34 (20.9%)	18 (17.6%)	8 (8.48%)
Nitrofurantoin	34 (I = 27) 20.9%	16 (I = 28) 15.7%	6 (I = 8) 12.5%
Penicillin	84 (51.9%)	46 (45.1%)	18 (37.5%)
Teicoplanin	10 (6.17%)	12 (9.8%)	8 (16.67%)
Tetracycline	132 (81.5%)	78 (76.5%)	38 (79.17%)
Vancomycin	22 (13.58%)	8 (7.84%)	4 (8.33%)

**Table 3: VRE isolates frequency from different clinical specimens**

Sample	SPECIES NAME			Total
	<i>E.faecalis</i>	<i>E.faecium</i>	<i>E.avium</i>	
Urine	14	6	4	24
Throat Swab	4	0	0	4
Abscess	2	0	0	2
Bile	2	2	0	4

**Table 4: VRE isolation in comparison to previous research [14-15]**

	Latika et al <sup>13</sup>	Present Study	Pol et al <sup>12</sup>
Sample size	92	312	172
VRE (%)	8 (8%)	34 (10.82%)	7 (4.06%)
Sample (positive)	Urine, Blood, CSF	Urine, Throat Swab, Abscess, Bile	Blood, Urine, Pus



Phenotype	Van A, Van B	Van A, Van B	Van A, Van M Van D
Minimum Inhibition Concentration value (µg/mL)	8 – 32	16-64	16 – 64

### 3. Discussion

Enterococci, are becoming significant pathogens associated with nosocomial infection worldwide. These bacteria are remarkable survivors because they can acquire genes encoding antibiotic hinderance in addition to a built-in resistance to a variety of anti-microbial agents and harsh conditions viz such as high temperatures, low pH, and high salinity<sup>13</sup>. Enterococcus is frequently isolated in clinical samples, in the present study 162 (51.29%) out of 312 were *E. faecalis* followed by *E. faecium* 102 (32.69%). This is similar to the study by Shah, L et al<sup>12</sup>, S. Farnandes et al.<sup>14</sup>, S. Bose et al<sup>15</sup>, Oberoi, L et al<sup>16</sup>, Karmarkar et al<sup>17</sup> but contradicts study conducted by authors in different part of India<sup>18-20</sup>. In the present study the resistance to high level gentamicin was found to be 59% and for Vancomycin the resistance is 11%, while the prevalence of Vancomycin reported is between 0 – 30%.<sup>21-25</sup> Almost 45% of all strains were resistance to Penicillin, which restricts the treatment choice as Penicillin combined with aminoglycosides were as a treatment choice. The mechanism involved may be low resistance binding protein or production of β-lactamase. The Penicillin resistance is similar to the study by Latika Shah.<sup>12</sup> A similar work was mentioned in the study conducted by Moosavian *et al.*,<sup>26</sup>. In divergence with the findings of the current investigation, the majority of the isolates of enterococci in another study by were *E. faecium*<sup>7,27</sup>.

Like Saeidi *et al.*, studies, the several enterococcal species in this investigation were identified from urine sample. Enterococci are common in urinary tract infections due to their dominance among the species isolated from urine samples<sup>4</sup>. After the urinary tract samples, it was the vaginal and throat swab samples from which the majority of the enterococci were isolated. This result was contradictory to the research conducted by Paul *et al*<sup>2</sup>. Though *E. faecium* and *E. faecalis* are more usually isolated, we isolated *E. avium* from the urine sample of hospitalized individuals, which is not often isolated from clinical specimens. *E. avium* was also

isolated from the urine samples in the research conducted by Bharti *et al*<sup>28</sup>.

The strongest resistance to ciprofloxacin, high-level gentamicin<sup>28</sup>, and tetracycline<sup>4</sup> was demonstrated by *E. faecalis*. Furthermore, the majority of *E. faecium* species showed hinderance to ciprofloxacin, tetracycline, and erythromycin. The current study's findings were in line with those of Saeidi *et al.*<sup>4</sup>. In the case of *E. avium* isolates, greater tetracycline antibiotic resistance was observed and an equal amount of antibiotic resistance was there for ciprofloxacin and erythromycin.

Vancomycin resistance was found to be 7.84% and 13.58% of *E. faecium* and *E. faecalis* respectively. In a study by Saeidi *et al.*, it was discovered that *E. faecalis* and *E. faecium* had a resistance of 3.16% and 26.68%, respectively in the case of vancomycin<sup>4</sup> which was contradictory to our findings. On comparing the vancomycin-resistant Enterococcus (VRE) with different studies it has been noted that the resistance pattern of Vancomycin is quite like the study conducted by Pol *et al* and his co-workers and Shah *et al*<sup>10-12</sup>.

In a current study, the resistance to vancomycin is 10% having both Van A and Van B phenotype resistance pattern. It was found that the resistance rate was 17% in 2013, 30% in 2014, and 18% in 2015. This shows that the resistance frequency of enterococcus to ciprofloxacin in the present work was equivalent with the trend as seen from 2013 to 2014. The detention rate of Enterococcus spp. to Vancomycin was 10% in the present study and it was 13% in 2013, 7% in 2014, and 16% in 2015. This shows that the Vancomycin resistance rate decreased in 2014 according to 2013 and again increased in 2015, but in the present study it has been found low in comparison to the data of 2013 and 2014<sup>3</sup>. Various factors like kind and quality of sample processed may be one of the reasons.

### Conclusion

In clinical sample *E. faecalis* is the predominant species, particularly isolated in cases of UTI and are in general resistant to three or more drugs. Resistance to



aminoglycosides and penicillin is an increasing concern of infection with Enterococci. With the emergence with Vancomycin resistance strains limits the therapeutic options for physicians. Since it is a nosocomial pathogen, strict hospital infection guidelines have to be implemented in hospitals which may help to restrict the infection caused by enterococci. Understanding the susceptibility patterns of enterococci is crucial for effective treatment. Routine screening helps identify infections caused by these bacteria, allowing clinicians to implement appropriate treatment plans. This approach is essential for managing life-threatening cases and mitigating antibiotic resistance.

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