



**Study of Phenotypic Identification and Antibiotic Sensitivity Pattern of Vancomycin Resistant Enterococci (VRE) Isolates From Different Clinical Samples of Patients in A Tertiary Care Hospital of Rajasthan**

<sup>1</sup>Dr. Subhash Saini, PG 3<sup>rd</sup> Year, Sardar Patel Medical College, Bikaner, Rajasthan

<sup>2</sup>Dr. Taruna Swami, Sardar Patel Medical College, Bikaner, Rajasthan

<sup>3</sup>Dr. Anshu Agrawal, Sardar Patel Medical College, Bikaner, Rajasthan

<sup>4</sup>Dr. Anjali Gupta, Sardar Patel Medical College, Bikaner, Rajasthan

**Corresponding Author:** Dr. Subhash Saini, PG 3<sup>rd</sup> Year, Sardar Patel Medical College, Bikaner, Rajasthan

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**Abstract**

**Background:** Enterococci are implicated to cause various community and hospital acquired infections, and Vancomycin-resistant Enterococci (VRE) are emerging as an important multi drug resistant pathogen causing nosocomial infections predominantly bacteremia and urinary tract infections.

**Materials and methods:** This is a prospective study. Enterococcal isolates obtained from various clinical specimens collected over a period of one year (1st May 2023 to 30th April 2024), are included in the study. Antimicrobial susceptibility testing (AST) was performed on Muller Hinton agar by Kirby-Bauer disk diffusion method and VRE detection was done by Vancomycin Agar Screen test, followed by microbroth dilution for MIC detection and Automation by Vitek-2.

**Result:** In the one year period; 500 Enterococci isolates were obtained from various clinical specimens. Out of these 76 (15.2%) isolates were found Vancomycin-resistant Enterococci (VRE). Most of these isolates were sensitive to Nitrofurantoin (96%), Linezolid (94.73%), Fosfomycin (94%), Teicoplanin (67.1%) and resistant to Ciprofloxacin (90.2%), Erythromycin (85%), Ampicillin (71.05%), High level Gentamicin (68%).

**Discussion:** In present study, we found 15.2% prevalence of VRE in Enterococcal isolates at our department; which is slightly high as compared to other studies.

**Conclusions:** The prevalence of VRE is on the rise in India. This increasing trend of VRE bacteremia and urinary tract infections is a red alert to the clinician and infection control practitioners, hence strict antibiotic

policies and strict IPC strategies are need of hour to reduce the VRE emergence in hospitals.

**Keywords:** Enterococcus, Vancomycin resistance, Nosocomial pathogen

### Introduction

Enterococcus is a Gram-positive organisms of ovoid shape found in pairs or short chains. Previously, they were classified as Streptococcus group D. In the early 1930s, enterococci were classified as group D streptococci and were differentiated from the non-enterococcal group D streptococci by distinctive biochemical characteristics. [1,2] Enterococci are part of the normal resident flora of the gastrointestinal tract of humans and animals. It is implicated to cause various community and hospital-acquired infections, such as endocarditis, bacteremia, meningitis, urinary tract infections and also associated with intra-abdominal infections. The WHO listed Vancomycin Resistant Enterococci (VRE) as a pathogen of high priority in its global list of important antibiotic-resistant bacteria. [3,4]

Of more than 50 species known, *Enterococcus faecalis* and *E. faecium* together account for the majority of (approximately 90%) clinical isolates (*E. faecalis* 80–85% and *E. faecium* 10–15%). Other less commonly isolated species include *E. gallinarum*, *E. casseliflavus*, *E. avium*, *E. durans*, *E. raffinosus*, *E. mundtii*, and *E. hirae*, accounting for approximately 5 to 10% infections. With the antimicrobial agents being frequently used in clinical treatment, antibiotic-resistant enterococci, particularly multi-drug resistant enterococci isolates, such as vancomycin-resistant enterococci (VRE) and linezolid resistant enterococci (LRE) have emerged and spread all over the world. [5,6] The first report of vancomycin resistant Enterococci (VRE) in India was done by Mathur in 1999, from New Delhi. Later, various

authors have reported prevalence of 1– 8.7% of VRE in India. Vancomycin was considered as one of the last lines of treatment against multidrug resistant organisms including ampicillin resistant enterococci and methicillin-resistant *Staphylococcus aureus* (MRSA). However, enterococci develop high level of resistance and the incidence of VRE infections among hospitalized patients has increased rapidly. [7,8]

The mechanisms of antibiotic resistance in enterococci are by mutation and horizontal gene transfer mediated via plasmids and transposons. Van A and van B genes are the most common phenotypes observed in hospital isolates. Enterococci containing van A gene, show high level resistance to vancomycin and teicoplanin antibiotics, while enterococci containing van B gene show low level resistance to vancomycin, but sensitive to teicoplanin. [9,10] VRE infections are associated with increased morbidity, mortality, and health care costs, and immunocompromised or critically ill patients are at risk of severe VRE infection. [11]

### Materials and Methods

#### Study Design and Period of Sampling

A cross-sectional study was performed in S.P. Medical College, Bikaner, over a period of One year (from May 2023 to April 2024). After obtaining clearance from the Institutional Ethics Committee, non-repetitive clinical specimens, such as blood, urine, cerebrospinal fluid (CSF), pus, tissue biopsy, catheter tips, vaginal swabs, and endotracheal aspirate were collected from both outpatients and inpatients, departments of institute, mainly from ICU, surgery, paediatric and urology wards are included in the study.

#### Screening and Identification of Enterococcus

All the collected clinical specimens were processed as per protocols, Direct Gram staining done and inoculated

onto blood agar and Mac Conkey agar using the streak plate method. All the plates were incubated at 37 °C for 48 h. The blood samples were inoculated into brain heart infusion broth and incubated at 37 °C for 24 h.<sup>[21]</sup> Identification was based on the typical magenta-colored colonies on the MacConkey agar, Gram positive cocci in pair or short chain from culture smear, catalase-negativity, growth on and blackening of bile-esculin agar, growth in the presence of 6.5% sodium chloride, heat tolerance test, motility testing, pigment production, and various biochemical tests including arginine dihydrolase test, carbohydrate fermentation test and pyruvate utilization test.<sup>[5]</sup>

#### **Antimicrobial susceptibility testing (AST)**

These isolates were tested for antimicrobial susceptibility to different antibiotics such as Ampicillin (10 µg), Tetracycline (30µg), Vancomycin (30µg), Teicoplanin (30µg), High level gentamicin (120µg) and Linezolid (30µg) for all isolates, whereas Ciprofloxacin (5µg), Fosfomycin (200µg) and Nitrofurantoin (300µg) were used for urinary isolates only, Erythromycin (15µg) and Doxycycline(30µg) are used for all non-urinary isolates.<sup>[1]</sup> The Kirby Bauer disk diffusion method was used to test the antimicrobial susceptibility of bacteria on Mueller Hinton agar plates using standard microbiological techniques as per Clinical and Laboratory Standards Institute (CLSI) guidelines. VITEK 2 Compact (BioMerieux Inc., France) was used to determine minimum inhibitory concentrations (MICs) for Benzyl penicillin, high level gentamicin, ciprofloxacin, levofloxacin, erythromycin, linezolid, teicoplanin, vancomycin, tetracycline, tigecycline and nitrofurantoin. An interpretation of susceptibility was performed according to the CLSI guidelines M100 33 edition.<sup>[18]</sup>

#### **Phenotypic Identification of Vancomycin-Resistant Enterococci (VRE)**

VRE resistance was determined by Agar Dilution Method with Vancomycin Hydrochloride powder and by Broth microdilution method on control cation adjusted Mueller Hinton Broth (CaMHB). For vancomycin agar screen test, first step is to Prepare the 1 mg/ml stock solution of vancomycin hydrochloride. Medium used for vancomycin agar screen is Brain Heart Infusion (BHI) agar supplemented with 6 µg/ml vancomycin. For the preparation of 100 ml of BHI agar plates containing 6 µg/ml vancomycin, take out of 600 µl of the 1 mg/ml vancomycin stock solution and add in 100 ml of autoclaved BHI media. (add the antibiotic stock while the autoclaved media is warm). The results were interpreted by CLSI guidelines M100 33<sup>rd</sup> edition. Enterococcus faecalis ATCC 29212 (vancomycin sensitive), E. faecalis ATCC 51299 (vancomycin-resistant) and Enterococcus faecium ATCC 700221 (vancomycin-resistant) were used as controls.<sup>[18]</sup>

#### **Results**

During a period of one year, 500 isolates of Enterococcus species were obtained from clinical specimens showing a prevalence rate of 15.2%. Among these 500 samples, there were 309 (61.8%) males and 191 (38.2%) females. Among these 500 isolates, 240 (48%) were under 18 years, 81 (16.2 %) were aged 19–40 years, 96(19.2 %) were aged 41-64 years, and 83 (16.6 %) were over 65 years of age. Among 500 Enterococcal isolates, most of them were obtained from Urine 371 (74.2%) followed by Blood 54 (10.8%), Sputum 25 (5%), Pus 15 (3%), CSF 10 (2%), BAL 7 (1.4%) and Others 18 (3.60%) [Table 1]. Among these 500 isolates of Enterococcus species 322 (64.4%) were Enterococcus faecalis, 143 (28.6%) Enterococcus

faecium and the others were Enterococcus avium, Enterococcus durans, Enterococcus casseliflavus and Enterococcus gallinarum [Table 2]. Among these, 76 (15.2%) were VRE (Vancomycin-resistant Enterococcus), In which 34(44.7%) isolates of Enterococcus faecalis, 25(32.8%) isolates of Enterococcus faecium, 2(2.6%) isolates of Enterococcus avium, 2(2.6%) isolates of Enterococcus durans and 6(7.8%) isolates of Enterococcus casseliflavus, 7(9.2%) isolates of Enterococcus gallinarum which were intrinsically resistant. Among these 76 VRE isolates, Enterococcus species were highly sensitive to linezolid, and teicoplanin. The isolates from urine samples were also highly sensitive to fosfomycin and nitrofurantoin. The sensitivity pattern of these VRE isolates is shown in Table3.

Table 1: Types of Specimens from which Enterococcus isolates

Specimen	Number (%)
Urine	371 (74.2%)
Blood	54 (10.8%)
Sputum	25 (5.0%)
Pus	15 (3.0%)
CSF	10 (2.0%)
BAL	7 (1.4%)
Others	18 (3.6%)
Total Enterococcus	500 (100%)

Table 2: Different Species of Vancomycin Resistant Enterococci (VRE) isolates

Enterococcus Species	Number	No. of VRE
Enterococcus faecalis	322	34
Enterococcus faecium	143	25
Enterococcus avium	8	2
Enterococcus durans	10	2
Enterococcus casseliflavus	6	6
Enterococcus gallinarum	7	7
Enterococcus hirae	2	-
Enterococcus cecorum	2	-
Total	500	76

Table 3: Antibiotic Sensitivity (%) pattern of these VRE isolates by Kirby-Bauer disc diffusion method (n=76)

Drug	Sensitivity (%)
Nitrofurantoin	96.0%
Linezolid	94.7%
Fosfomycin	94.0%
Teicoplanin	67.1%
Doxycycline	42.3%
High level gentamicin	32.0%
Ampicillin	28.9%
Tetracycline	26.3%
Ciprofloxacin	3.9%
Erythromycin	-

### Discussion

The prevalence of VRE infections in India is increasing in the past one decade. Mathur P et al., from New Delhi was the first to report VRE from India in 1999. [23] The reports of prevalence of VRE from India vary from 1% to 8.7%. In the present study, a total of 76 (15.2%) VRE isolates were obtained and rate is high as compared to other Indian studies, as a study performed by Taneja et al., in Chandigarh (2004), the prevalence of VRE isolates was found to be 5.5% and another study performed by Praharaj et al., in Puducherry (2008), there was a prevalence of 8.7% VRE isolates from all clinical Specimens. [22,27]

In the present study, maximum number of Enterococci were isolated from urine specimen 371 (74.2%), Because most Specimen were collected from Urology department, which is higher than Praharaj et al., (59.1%) and Sengupta et al., (60.1%). [22, 1] In the present study, among these 500 Enterococcal isolates, there were 322 (64.4%) Enterococcus faecalis, 143 (28.6%) Enterococcus faecium, 10 (2.0%) Enterococcus durans, 8 (1.6%) Enterococcus avium, 7 (1.4%) Enterococcus gallinarum, 6 (1.2%) Enterococcus casseliflavus. In a

similar study performed by Sengupta et al., *E. faecalis* was the most common isolated species 64.42%, followed by *Enterococcus faecium* 30.72%, *Enterococcus avium* 2.16%, *Enterococcus durans* 1.08%, *Enterococcus casseliflavus* 1.08%, and *Enterococcus gallinarum* 0.54%.<sup>[1]</sup> Another study in Uttar Pradesh have shown similar results, (47 *E. faecalis*, 51 were *E. faecium*, two were *E. gallinarum* and one was *E. casseliflavus*).<sup>[28]</sup> The recent studies have shown an increase in the isolation rate of *E. faecium* and other non -*faecalis* species of *Enterococcus*.<sup>[25]</sup>

In our study, among these 76 VRE isolates, *Enterococcus* species were highly sensitive to linezolid, and teicoplanin. The isolates from urine samples were also highly sensitive to fosfomycin and nitrofurantoin and highly resistant to Ciprofloxacin, Erythromycin, Ampicillin and High-Level Gentamicin (HLG). Nitrofurantoin, found to be highly sensitive to VRE isolates about 96.0% and Ciprofloxacin, found to be highly resistance to VRE isolates about 90.2%. 5.6% of the *Enterococcus*, which showed intermediate sensitivity to vancomycin by the Kirby Bauer disc diffusion method, was further tested by the Vancomycin Agar Screen Test, in which 2.6% *Enterococcus* isolates were found to be sensitive and 3.0% *Enterococcus* isolates were found to be resistant to vancomycin by the Vancomycin Agar Screen Test.

### Conclusion

The vancomycin resistance rate among the *Enterococcus* isolates was 15.2% in our study which is high as compared to other reports from North India. Presence of vancomycin-resistant *E. faecalis* and *E. faecium* strains could be an alarming situation in hospital settings. This increasing trend of VRE infections is a red alert to the clinicians, and strict antibiotic policies and proper

adherence to the infection control practices should be followed to reduce the VRE rate. It is very important to identify, treat, and take preventive measures to limit the spread of VRE, which otherwise will result in serious consequences.

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