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# PREVALENCE OF VANCOMYCIN-RESISTANT ENTEROCOCCUS ISOLATED FROM CLINICAL SPECIMENS AT TERTIARY CARE HOSPITAL

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#### **Abstract:**

**Introduction:** Vancomycin-resistant enterococci (VRE) has been recognized as a serious healthcare problem in recent times. Understanding the exact prevalence rate, and virulence factors among enterococci isolated from various clinical specimens is essential for controlling the spread of bacterial resistance and for epidemiological surveillance within the local healthcare settings. So, this study was performed to find out the prevalence of VRE and related risk factors among clinical specimens in the institute.

**Material & Methods:** This study was carried out on enterococci isolated from clinical specimens sent to the microbiology laboratory of the institute e.g. blood, urine, pus, wound discharge, CSF, and other body fluids (pleural and peritoneal). Direct microscopic examination was done using wet film for urine specimens and Gram stain for other specimens like pus and body fluids, looking for leukocytes and bacterial cells.

Results: Maximum isolates (70.59%) had shown VanA type resistance with high-level vancomycin resistance (MIC >256 µg/mL) while 17.65% of VRE isolates expressed VanB phenotype having moderate to high-level resistance to vancomycin and sensitive to teicoplanin. The rate of VRE infection was found to be significantly higher among patients who underwent surgical intervention. Among hospital-related risk factors, hospital stay(≥48hrs), ventilation, central venous catheterization, and admission to ICU were significantly associated with VRE infection. (p value<0.05). Conclusion: With a steady worldwide spread VRE can be expected as a major challenge to healthcare authorities in upcoming years. A multi-disciplinary approach is urgently needed including regular surveillance for the local epidemiology, early detection, and management, especially in the face of high-risk settings.

#### INTRODUCTION

Enterococcus, a part of normal human intestinal flora, also present in less numbers in oral cavity, genitourinary tract and perianal skin, has evolved over the past century from being a commensal of little clinical significance to becoming the second most common nosocomial pathogen. [1,2] Enterococci are capable of causing various infections usually hospital acquired, such as endocarditis, bacteraemia, urinary tract infections (UTIs), surgical wound infections, intra-abdominal and pelvic infections, neonatal sepsis and rarely central nervous system infections. [3,4]

Over the years enterococci have become increasingly resistant to antibiotics in terms of both multiplicity of resistance and level of resistance to a particular drug.<sup>5</sup> Vancomycin resistant enterococci (VRE) has been recognised as a serious health care problem in the recent times, especially in critically ill patients with limited therapeutic choices. [5]

### Risk Factors for emergence of VRE- [6,7]

- Prolonged stay in an ICU or hospital, in particular immunosuppressed ICU population who have been given vancomycin, third-generation cephalosporins, and/or antibiotics with activity against anaerobes.
- Exposure to other patients with VRE either by close proximity to a VRE-colonized patient, or by care from a nurse providing who is care-giver to another VRE-colonized patient.
- Certain patient populations, notably those on chronic hemodialysis, with hematological malignancies, or undergoing liver transplantation, patients with neutropenia, etc.
- Other factors which may increase the risk include bladder catheterization, expression of cytolysin as an enterococcal virulence determinant.
- Companion animals and pets can also be a reservoir for VRE.

The overall national data of VRE infection rate is yet to be available from India due to paucity of cumulative data collection system. Understanding the exact prevalence rate, virulence factors and monitoring antimicrobial resistance pattern among enterococci isolated from various clinical specimens is essential for controlling the spread of bacterial resistance and important for epidemiological surveillance within the local healthcare settings. With this background keep in mind study was planned with objectives to find out the prevalence of VRE and related risk factors among enterococci clinical specimens because it is crucial to suspect enterococcal infection in the face of risk factors for better prevention and early management.

### **MATERIALS AND METHODS:**

**Study Area:** This observational study was carried out at tertiary care teaching hospital and Training center, Central India. The study commenced after approval from the institutional ethical committee.

**Selection criteria**: Enterococci isolated from Clinical specimens sent to the microbiology laboratory of the institute were included in study e.g. bood, urine, pus, wound discharge, CSF and other body fluids (pleural and peritoneal).

### **Exclusion criteria:**

Enterococcal isolates from stool, respiratory tract specimens and vaginal swabs were excluded as colonizer.

### **Methodology:**

Informed verbal consent has been taken from chosen all study patients by explaining the purpose. Enterococcal isolates from stool, respiratory tract specimens and vaginal swabs were excluded as colonizer. Specimens were processed according to standard procedures. Direct microscopic examination was done using wet film for urine specimens and Gram's stain [8] for other specimens like pus and body fluids, looking for the presence of leukocytes and bacterial cells. Inoculation was done on blood agar and MacConkey agar. Additionally chocolate agar was also inoculated for CSF

specimens. Isolates from clinical specimens with a suggestive colony morphology and Gram's stain appearance that of *Enterococcus spp* and a negative catalase reaction were included and processed. Further speciation and species confirmation was done by Vitek 2 system. [9] MIC determination by Vitek2 system<sup>49</sup> was done for all antimicrobials using susceptibility card for gram+ve organisms and interpreted according to CLSI guidelines 2018.

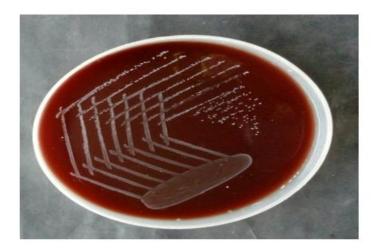


Image 1: Colonies of Enterococcus on blood agar

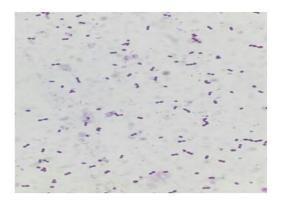


Image 2: Gram stain of culture smear from *Enterococcus* colonies showing gram +ve ovoid cocci in pairs and short chains



**Image 3:** Vitek 2 System (left) and GP cards with cassette (right)

Depending on the MIC levels for vancomycin and teicoplanin and *Enterococcus spp*, the three Van resistance phenotypes were determined as following. [10]

a) VanA- vancomycin MIC; 64-1000 μg/mL, teicoplanin MIC;16-512 μg/mL

- b) VanB- vancomycin MIC;4-1000 µg/mL, teicoplanin MIC;0.5 to 1 µg/mL
- c) VanC- vancomycin MIC;2-32 μg/mL, teicoplanin MIC;0.5 to 1 μg/mL, in intrinsically vancomycin resistant species like *E. gallinarum*

In addition, in this study the detailed history was taken as per the case report form to find out any risk factors associated with vancomycin resistance with special reference to-

#### I.Host related:

- Decreased host defences & chronically debilitated host (viz. malignancy and administration of chemotherapy, HIV infection, Tuberculosis, Diabetes, Renal failure, Cirrhosis, prolonged corticosteroid therapy etc.)
- Surgical intervention
- II. Hospital related
- Prolonged(≥48hrs) and previous hospitalization
- Ventilation
- Central venous catheter
- Urinary catheter
- ICU admission

### III.Antibiotic related

• Prolonged use of broad spectrum empirical antibiotics before isolation of *Enterococcus* especially vancomycin and to which enterococci are intrinsically resistant (viz. cephalosporins, cotrimoxazole, clindamycin etc.)

### **Data analysis:**

The response sheets were retrieved and entered in MS Excel for further analysis by using Epi-info 3.01 version software. The frequency presentation has been done in the form of tables and graphs. Categorical variables were analysed using Pearson's Chi-square test and Fischer exact test. P value <0.05 was taken as statistically significant.

### **OBSERVATION & RESULTS:**

### **Socio-demographic characteristics:**

A total of 123 enterococcal isolates were obtained from patients with variety of enterococcal infections. Table 1 shows demographic characteristics of patients with enterococcal infections. Males were slightly more with a M: F ratio of 1.32:1. Average age of study patients was 31.35yrs. Maximum number of patients were in 21-30yrs age group at 21.14% followed by 31-40yrs at 19.51%.

Enterococcal isolates were obtained from a variety of clinical specimens such as urine, blood, pus, CSF (cerebrospinal fluid) and other body fluids like pleural & peritoneal fluid. The distribution of enterococci among different clinical specimens was observed and found that maximum isolates were obtained from urine (50.41%) followed by blood (26.83%) while 15.45%, 3.25% and 4.06% from pus, CSF and other body fluids respectively. [Table 2]

<b>Table 1:</b> Demographic characteristics of patients with enterococcal infection	ns
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Variables	Category	Frequency (Percent)
Sex	Male	70 (56.91%)
	Female	53 (43.09%)
Age	≤10yrs	23 (18.70%)
	11-20yrs	13 (10.57%)
	21-30yrs	26 (21.14%)
	31-40yrs	24 (19.51%)
	41-50yrs	18 (14.63%)
	51-60yrs	10 (8.13%)
	>60yrs	09 (7.32%)
	Total	123 (100%)

Table 2: Distribution	of enterococci	among different	clinical specimens

Specimen	Number of isolates (%)
Urine	62 (50.41%)
Blood	33 (26.83%)
Pus	19 (15.45%)
CSF	4 (3.25%)
Other body fluids	5 (4.06%)
Total	123 (100%)

Figure 1 shows distribution of enterococcal isolates among patients admitted to different hospital units. Maximum isolates were recovered from medical wards (adult and paediatric wards), being 43.90%, while almost equal numbers from surgical wards and intensive care unit (ICU) i.e. 28.46% and 27.64% respectively. ICU here includes medical, surgical, paediatric and neonatal intensive care units.

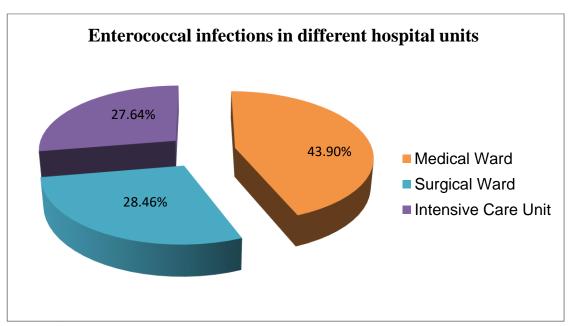


Figure 1: Distribution of enterococcal infections in different hospital units

On considering the specimen-wise distribution of enterococcal species different species were isolated from all specimens except *E. gallinarum* which was isolated only from pus and CSF specimens with one isolate from each respectively.

Most common species among urine, pus and other body fluids was *E. faecalis* constituting 74.19%, 63.16% and 80% of these isolates respectively while in blood and CSF more common species was *E. faecium* being 51.52% and 75% respectively. *E. faecalis* was not isolated from CSF.

**Table 3:** Distribution of enterococcal species among different clinical specimens

Specimen	Enterococcus faecalis	Enterococcus faecium	Enterococcus gallinarum
Urine (n=62)	46 (74.19%)	16 (25.81%)	-
Blood (n=33)	16 (48.48%)	17 (51.52%)	-
Pus (n=19)	12 (63.16%)	6 (31.58%)	1 (5.26%)
CSF (n=4)	-	3 (75%)	1 (25%)
Other body fluids (n=5)	4 (80%)	1 (20%)	-

# Prevalence of VRE among enterococci from clinical specimens:

MIC for vancomycin was determined by E-test and Vitek2 system and vancomycin resistance was detected in 17 (13.82%) out of total 123 enterococcal isolates. M:F ratio among VRE infected patients

was 1.83:1. It affected all ages ranging from 10 days to 65 years, while maximum i.e. 5 patients belonging to age group  $\leq$ 10 years, followed by 4 falling in 21-30 years age group.

According to the MIC values for vancomycin (VA) and teicoplanin (TEI), resistance phenotype was determined. Maximum isolates (70.59%) had shown VanA type resistance with high level vancomycin resistance (MIC >256  $\mu$ g/mL) while 17.65% of VRE isolates expressed VanB phenotype having moderate to high level resistance to vancomycin and being sensitive to teicoplanin. [Table 4]

<b>Table 4:</b> Distribution of different	vancomycin resistant	phenotypes among V	VRE isolates (n=17)

Phenotype	n (%)	VA MIC (µg/mL)	TEI MIC (µg/mL)
VanA	12 (70.59%)	>256	>16 (n=2)
			>32 (n=10)
VanB	03 (17.65%)	>32 (n=2)	0.5-1.0
		>256 (n=1)	
VanC	02 (11.76%)	>16	0.5-1.0

**Table 5:** Prevalence of VRE among different clinical specimens

Specimen	n (%)
Urine (n=62)	2 (3.23%)
Blood (n=33)	9 (27.27%)
Pus (n=19)	3 (15.79%)
CSF (n=04)	2 (50%)
Other body fluids (n=05)	1 (20%)

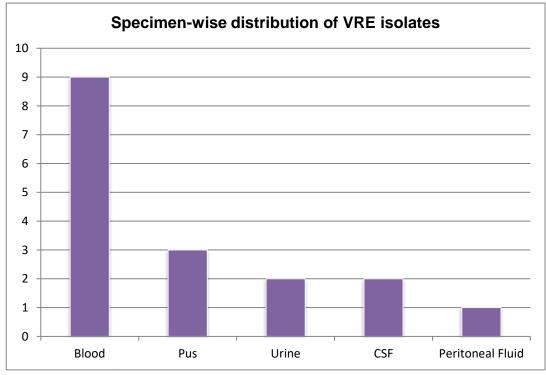


Figure 2: Specimen-wise distribution of VRE isolates

Vancomycin resistance was found in isolates obtained from all clinical specimens with maximum prevalence among CSF isolates being 50% followed by blood isolates showing 27.27% resistance. Least resistance was shown by urinary isolates at 3.23%. [Table 5, Figure 2] VRE was isolated from patients admitted to different hospital units, however maximum rate of VRE was found among ICU admitted patients with 38.24% of total ICU patients with enterococcal infection being vancomycin resistant.

# Related risk factors among patients with VRE and VSE infection:

Table 6 shows frequency of risk factors among patients with VRE and VSE infection. Rate of VRE infection was found to be significantly higher among patients who underwent surgical intervention. (p value=0.007417) Among hospital related risk factors, hospital stay(≥48hrs), previous or multiple hospitalizations, ventilation, central venous catheterization and admission to ICU were significantly associated with VRE infection. (p value<0.05)

Among these factors, increased rate of VRE infection associated with hospital stay(≥48hrs), and ICU admission was found to be highly significant. (p value<0.001) Use of 3<sup>rd</sup> generation cephalosporins and vancomycin was also significantly associated with higher rates of VRE infection. (p value<0.05)

**Table 6:** Frequency of risk factors among patients with vancomycin resistant *Enterococcus* (VRE) and vancomycin sensitive enterococcus (VSE) infection

Host-related	VRE (n=17)	<b>VSE</b> (n-106)	P value
Malignancy	3 (17.65%)	5 (4.72%)	0.1592*
Diabetes	3 (17.65%)	20 (18.87%)	>0.9999*
Tuberculosis	1 (5.88%)	3 (2.83%)	$0.9056^*$
Renal insufficiency	3 (17.65%)	11 (10.38%)	0.5999*
Cirrhosis & liver failure	1 (5.88%)	4 (3.77%)	>0.9999*
Prolonged corticosteroid use	1 (5.88%)	7 (6.60%)	>0.9999*
Heart failure	2 (11.76%)	5 (4.72%)	$0.4972^*$
Surgical intervention	8 (47.06%)	15 (14.15%)	$0.007417^*$
Hospital-related			
Hospital stay(≥48hrs)	15 (88.23%)	42 (39.62%)	$0.0001904^{**}$
Previous/multiple hospitalizations	8 (47.06%)	16 (15.09%)	$0.01027^*$
Ventilation	7 (41.18%)	17 (16.04%)	$0.04647^*$
Central venous catheter	6 (35.29%)	11 (10.38%)	$0.02784^*$
Urinary catheterization	8 (47.06%)	48 (45.28%)	0.8914**
ICU admission	13 (76.47%)	21 (19.81%)	$0.00001632^*$
Antibiotic usage-related			
Multiple empirical antibiotics	4 (23.53%)	21 (19.81%)	0.9385*
3 <sup>rd</sup> generation cephalosporins	13 (76.47%)	51 (48.11%)	0.02981**
Vancomycin	8 (47.06%)	19 (17.92%)	$0.02426^*$

Enterococcus being considered Innocuous commensal of human and animal gastrointestinal tract for long periods of time, it have come to hold the position of one of the most important nosocomial pathogen over the last few years, ranked as second most common cause of healthcare associated infections according to National Healthcare Safety Network of the Centers for Disease Control and Prevention. Impact of nosocomial infections is enormous regarding morbidity, mortality and economic loss.

### **DISCUSSION:**

Enterococcus being considered Innocuous commensal of human and animal gastrointestinal tract for long periods of time, it have come to hold the position of one of the most important nosocomial pathogen over the last few years, ranked as second most common cause of healthcare associated infections according to National Healthcare Safety Network of the Centers for Disease Control and Prevention. Recently, enterococci have become organisms of increased interest amongst healthcare associated infections owing not only to their ability to cause serious infections and to survive in hospital environment for long time periods, but also because of their intrinsic resistance to many antibiotics and increasing rates of acquired resistance to commonly used antibiotics. Nosocomial transmission of enterococci can take place by direct or indirect modes like feco-oral route and invasive devices harbouring colonization by this flora. [8]

In this study, we isolated 123 consecutive enterococcal isolates from clinical specimens obtained from patients with a variety of enterococcal infections admitted in different wards and intensive care units of our institution. Study subjects were constituted by slightly more number of males (56.91%) with a M:F ratio of 1.32:1. Some studies have reported male preponderance [11,12], while female preponderance have also been reported. [13,14] The reason for predominance of male patients can be due to the fact that, it is generally the males who seek medical attention as compared to females who tend to ignore their illnesses. Also, it depends upon the composition of study patients and patient population seeking particular healthcare facility. [15]

Maximum number of patients belonged to the age group of 21-30yrs at 21.14%. Unlike many studies where enterococcal infections were not commonly reported from paediatric patients, significant number (18.70%) of our patients belonged to ≤10yrs age group. Similarly, **Yadav G** *et al.* [16] has reported maximum patients (23.5%) in 20-29yrs age group and 11% of paediatric patients. **Yilema A** *et al.* [17] reported median age of study subjects at 20yrs and found that difference in age was not significantly associated with enterococcal infection while paediatric patients were very high in number at 50%. This variation in age distribution of study participants can be explained by varied composition of study population and also difference in frequency of infections at different body sites which may have certain age predilection.

Nosocomial UTI is the most common infection caused by these organisms. In our study, enterococcal isolates were obtained from a variety of clinical specimens with maximum (50.41%) from urine followed by blood (26.83%). Pus and exudates grew 15.45% of isolates and 4 (3.25%) strains were also isolated from CSF. Various studies corroborated our findings with maximum clinical enterococcal isolation from urine samples but second most common infection noted in their study was wound infection and isolation rate from blood was lesser being reported as 2%, 7%, 13.6% and 3.74%.[18,19]

In our study *E. faecalis* was the predominant speices being isolated at a rate of 63.41% followed by *E. faecium* (34.96%). The similar species distribution was shown by many Indian studies with isolation rates of *E. faecalis* ranging from 56% to 79.44% and that of *E. faecium* ranging from 20.56% to 34%. However a few reported *E. faecium* as predominant species. <sup>51</sup> However gradual increase in *E. faecium* isolation over that of *E. faecalis* can be explained by selecting up of *E. faecium* after eliminating *E. faecalis* as the earlier is more resistant to commonly used enterococcal antibiotics. [20,21]

At our centre we detected vancomycin resistance in 17 (13.82%) out of total 123 enterococcal isolates. The prevalence rates of VRE reported by various Indian researchers fall between 0 and 25.2%. [22-25] The differences in the prevalence of VRE in different regions are governed by various factors including use of glycopeptides in humans and animals (growth promoters). Documented VRE prevalence rates by Indian studies vary as 8.72% from Pondicherry [6], 5.76% from Salem [1], 7.9% from Uttar Pradesh [5], and 7% from Kolkata [2]. The difference in the published rates of VRE infection may reflect differences in the infection control practices, antibiotic consumption policies, cultural differences among health care personnel.

Rate of VRE infection was found to be significantly higher among patients who underwent surgical intervention, who stayed in the hospital for ≥48hrs, who had history of previous or multiple hospitalizations, who had been put on ventilation or who underwent central venous catheterization and those who were admitted to ICUs were significantly associated with VRE infection. (p value<0.05) Among these factors, increased rate of VRE infection associated with hospital stay(≥48hrs), and ICU admission was found to be highly significant. (p value<0.001) Use of 3<sup>rd</sup> generation cephalosporins and vancomycin was also significantly associated with higher rates of VRE infection. (p value<0.05)

Our findings were corroborated by Tripathi A *et al.* [5] who also found surgical procedure, hospitalization for  $\geq$ 48hrs, ICU admission, use of invasive devices such as ventilator and central

venous catheter as significantly associated with VRE infection. However, they also found renal sufficiency significantly associated with VRE infection but in our study, it was not statistically significant. Other authors like Monteserin N *et al.* [10] found ICU stay, prior hospitalization, renal failure, malignancy and antibiotic usage as risk factors for VRE infection.

### **CONCLUSION:**

With a steady worldwide spread VRE can be expected as a major challenge to healthcare authorities in the coming years. Owing to its propensity to affect debilitated patients in critical care units which are exposed to multiple empirical antimicrobials with frequent use of invasive devices and its ability to survive in hospital environments for longer duration, it becomes difficult to be eradicated. Having limited therapeutic choices left, VRE can cause significant morbidity and mortality. A multi-disciplinary approach including regular surveillance for the local epidemiology, early detection and management especially in the face of high risk settings, implementation and strict compliance to infection control practices and antimicrobial stewardship, collaboration between treating clinician and microbiologists for timely de-escalation of empirical antimicrobials to avoid overuse is a need of time to prevent this infection and to curtail its intra- and inter-hospital, so the global transmission.

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