



## Original Research Article

## A descriptive study of vancomycin resistant enterococci in Tertiary Care Hospital of Western India

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## ABSTRACT

**Background and Aims:** Enterococci are second most commonly isolated hospital-acquired pathogen from urinary tract and wounds. Being notorious organism enterococci has presented treatment challenges resulted in newer drug development followed by resistance development in segments; such as Vancomycin. Present study aims at detecting prevalence, distribution and susceptibility patterns of Vancomycin Resistant Enterococci (VRE) found in clinical isolates.

**Materials and Methods:** A descriptive study was carried out in the Microbiology department of Tertiary care hospital, Western India including a total of 36,027 clinical isolates received in the duration over 2 years from indoor patients across disciplines. Enterococci were recognized by standard biochemical tests. Antimicrobial susceptibility testing was done as per CLSI guideline. VRE was distinguished by disc diffusion method and Minimum Inhibitory Concentration Test. Qualitative data were presented as proportions.

**Results:** On susceptibility testing, the prevalence of VRE was found out to be 11.13%. Maximum number of VRE isolates were from Urine (49.06%), followed by blood culture (32.08%), and swab (5.66%). Among VRE; 56.6% isolates were *E. Faecium* followed by other enterococcus (35.85%) and *E. Faecalis* (7.55%) respectively. Highest resistance was found for penicillin, ampicillin and levofloxacin, while most sensitive were linezolid and fosfomycin.

**Conclusion:** Considering versatile ability to advance and transmit antimicrobial resistance, VRE represents a bottle neck in treatment strategies. Rational prescription of antibiotic, VRE surveillance and timely antibiogram in admitted cases is the need of hour.

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### 1. Introduction

Enterococci are gram-positive anaerobes that live as commensal habitant in the alimentary canal of a person.<sup>1</sup> Enterococci have been perceived as a predominant reason for endocarditis for very nearly a century. Notwithstanding this since a long time, enterococci started to be perceived as normal cause for hospital acquired diseases in the 70s and have developed as genuine nosocomial pathogens liable for contaminations of the urinary tract, circulatory system, meninges, wounds and the biliary tract. They are now ascendant nosocomial pathogens; having become the second most

commonly isolated pathogen from urinary tract and wound contaminations and the third most common cause of nosocomial septicemia.<sup>2</sup> Reported ascend in predominance of enterococcal diseases in people are impacted somewhat by the capacity of enterococci to escape from the activity of regularly utilized disinfectants. Alongside expanding antimicrobial opposition, the procurement of harmful factors and the capacity of enterococcus to frame bio-layers have additionally added to the ascent in nosocomial prevalence.<sup>3</sup>

Treatment challenges introduced by enterococci were very much perceived as long as since 50s, when improvement from enterococcal endocarditis to penicillin utilized alone were seen as notably lower than those

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of streptococcal endocarditis. Moulded by the specific weights of their serious condition, enterococci have advanced a various cluster of reactions and hereditary versatility permitting them to flourish in the human biological advances. Research and development in current medication have expanded the capacity to support life in basically critical patients expanding the vulnerability to contamination and bringing about fast patient turnover and broad utilization of anti-toxins.<sup>1</sup>

A standard regimen of penicillin and gentamicin had been the backbone of treatment for enterococcal contaminations till date however with the rise of elevated level aminoglycoside resistance (HLAR), vancomycin is the main elective left. Vancomycin had been in clinical use for over 30 years without the rise of stamped resistance. Since rise of vancomycin resistant enterococci (VRE) from patients in the Britain and France, in limited time it gained access across the globe.<sup>2</sup> There is paucity of database related to VRE in developing countries of South East Asia particularly India. In this study with the help of retrospective analysis of a database in tertiary care centre over western India, we studied prevalence, distribution and antibiotic susceptibility patterns of VRE found in various clinical isolates.

## 2. Materials and Methods

A descriptive study was carried out in the microbiology department of tertiary care hospital, Western India. A total of 36,027 samples received in the duration over 2 years from January 2017 to December 2018 at microbiology department of tertiary care hospital were assessed.

### 2.1. Clinical isolates

The various clinical isolates were blood, urine, pleural fluid, cerebrospinal fluid, pus, ascetic fluid, etc. All received isolates from patients admitted in various wards like; surgery, medicine, orthopaedics, gynaecology, paediatrics, etc. in mentioned study duration of 2 years were included as a part to retrospective data analysis. There were such 36,027 clinical isolates which have been identified as sample and analyzed. The samples were processed for microscopy, culture and sensitivity testing according to standardized laboratory protocols.

### 2.2. Specimen processing

Enterococci were recognized by standard biochemical tests like; bile esculin hydrolysis test, arabinose fermentation test and affirmed by optochin and bacitracin plate test. Bile esculin test helps in differentiating group D streptococci from other streptococci. Group D streptococci have peculiar ability to hydrolyze esculin in availability of bile salts. Anti-microbial susceptibility testing was finished by Kirby bauer plate dispersion strategy on Muller hinton agar as indicated

by standard CLSI guideline. Vancomycin resistance was distinguished by disc diffusion method and affirmed by Minimum Inhibitory Concentration Test (MIC).<sup>4</sup>

### 2.3. Identification of enterococci

Enterococci are identified microscopically by their distinguished characteristics in Gram stain. In microscopic examination they are found in pairs and at obtuse angles and later catalase test are done. Catalase test helps in primary differentiation of enterococci from staphylococci. Staphylococci are gram positive where as enterococci are gram negative.<sup>4</sup>

The further speciation was done by subjecting the isolates with a panel of biochemical and various tests like sugar fermentation (Glucose, Sucrose, Mannitol, and Arabinose) and was subjected to motility for result interpretation. A specific carbohydrate containing basal medium was used for sugar fermentation. A production of acid carbohydrate complex helped in identification of enterococcus. Appearance of yellow colour indicated acid production and was considered as positive response. Appearance of reddish pink colour indicated negative result. In negative result medium remained purple. Appearance of orange colour indicated delayed response. In such scenarios after comparing with un-inoculated tube re-incubation was carried out.<sup>4</sup>

### 2.4. Anti microbial susceptibility testing of enterococcal isolates

All the enterococcal species were subjected to modified Kirby bauer plate diffusion as per standard procedure suggested by CLSI guidelines. Antibiotic susceptibility testing was done by Kirby-Bauer disk diffusion method using antibiotic disks and Mueller Hinton Agar as per formed protocol by CLSI.<sup>4</sup>

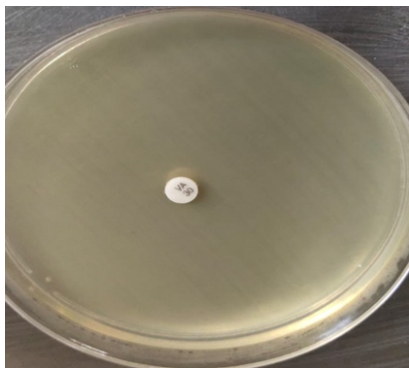
Vancomycin was used for detection of vancomycin resistance. After complete 24 hour of incubation the Mueller Hinton agar containing the vancomycin antibiotic was observed with naked eye using transmitted light for presence or absence of zone of inhibition around the disk. On presence; Inhibition zones, were calculated with ruler. Any identified growth within the zone of inhibition was also observed.

Ethical approval was obtained from institutional ethical committee.

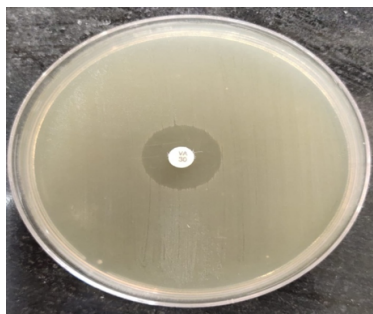
### 2.5. Statistical analysis

Study was done using retrospective data collected over two years of duration. All data are expressed as absolute numbers and percentage to serve the purpose of descriptive analysis in current study. For diagrammatic presentation of qualitative data; pie chart was used. Other categorical data were presented in tabular form along with frequency and

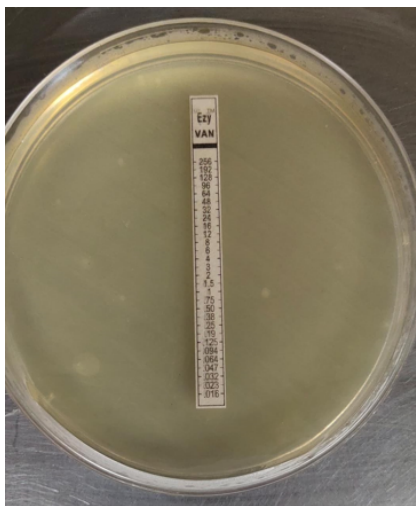
percentage distribution. Data were entered and analyzed using MS office Excel and Epi Info, CDC, Atlanta for statistical inference.



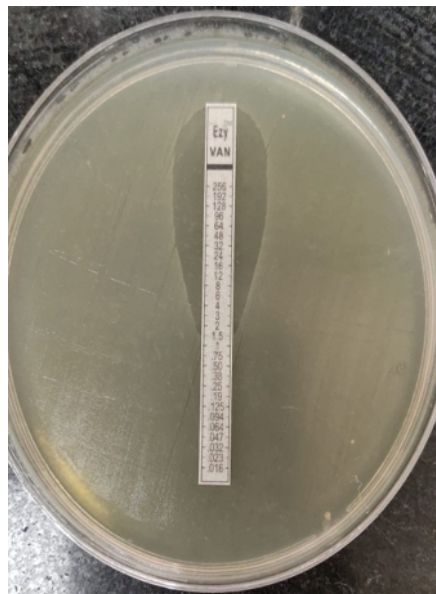
**Fig. 1:** Enterococcal strain showing resistance to vancomycin (30 mcg) disk



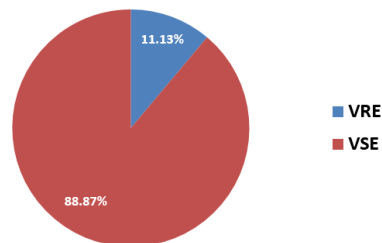
**Fig. 2:** Enterococcal strain showing susceptibility to vancomycin (30 mcg) disk



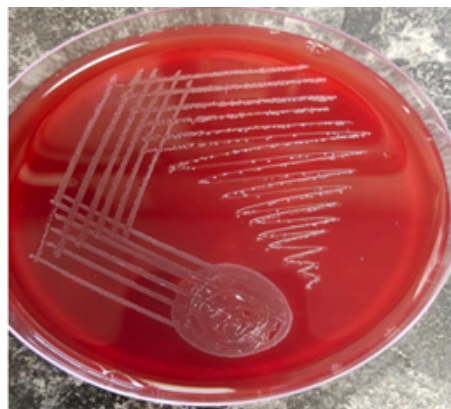
**Fig. 3:** Enterococcal strain showing resistance to vancomycin MIC > 256 µg



**Fig. 4:** Enterococcal strain showing susceptibility to vancomycin MIC=1.5 µg



**Fig. 5:** Distribution of enterococcus according to vancomycin resistance



**Fig. 6:** Colonies of enterococci on blood agar

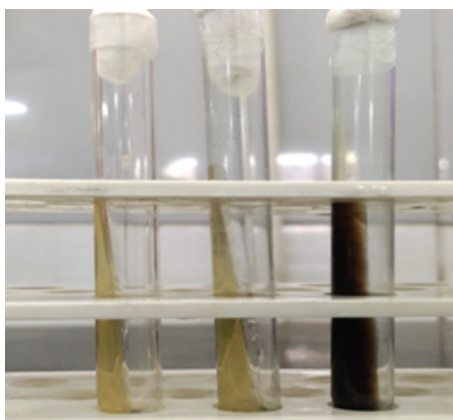


Fig. 7: Bile esculin agar test

### 3. Results

A total of 36,027 clinical isolates were analyzed at Microbiology department of Government medical college, Western India during time period of 2 years (2017-18). Among these clinical isolates; 476 enterococcus species were grown in culture with prevalence rate of 1.32%. On susceptibility testing, the prevalence of Vancomycin resistant enterococcus was found out to be 11.13% (53 isolates of VRE) among various enterococcus species. Distribution of the same is described in (Figure 5).

Among VRE; 56.6% isolates were accounting to *E. Faecium* followed by other enterococcus species (35.85%) followed by *E. Faecalis* (7.55%). On looking in to gender perspective; 52.83% of male and 47.17% of females were reported with VRE. The median age of the cases was 24 years (Range: 2 Days — 70 years).

(Table 2): Describes distribution of enterococcus and vancomycin resistant enterococcus in contexts of isolate and concerned department of isolation. Maximum number of enterococcus isolates were from Urine (65.97%), followed by blood culture (11.13%), swab (10.50%) and others. Similar pattern was observed in VRE isolates; i.e. 49.06% from Urine, followed by 32.08% from blood culture, and 5.66% from swab followed by others.

(Table 3): Depicts the antimicrobial resistance patterns of VRE found among clinical isolates. Highest resistance was found for commonly used anti-microbial agents like; penicillin, Ampicillin and levofloxacin. 100% resistance was found for all these 3 agents across all species of VRE. Most sensitive anti-microbial agents were linezolid and fosfomicin.

### 4. Discussion

Vancomycin resistant enterococci have been increasingly reported worldwide since its first description in 1987.<sup>5</sup> The present study documents prevalence, distribution and susceptibility patterns of enterococcus and VRE over a

period of two years at Government medical college, Western India.

Determining the prevalence of antibiotic resistant organism is an important step in the formulation of interventions to control emergence and transmission of resistant pathogens.<sup>6</sup> In present study the prevalence rate of enterococcus was found out to be 1.32% while the prevalence of VRE was found out to be 11.13%. In a study done by Sreeja S. et al.,<sup>6</sup> the prevalence rate of enterococcus was found out to be 2.3%. In a systematic review and meta-analysis done by Melese A. et al. which included 20 studies across PubMed, EMBASE, Google scholar, African Journals Online (AJOL), etc. recorded pooled prevalence of VRE as 14.8%. The pooled prevalence of VRE in the sensitivity analysis ranged from 13.2 to 16.7%.<sup>7</sup> The VRE prevalence of 7.9% was recorded in a study done at tertiary care hospital in Northern India.<sup>8</sup>

Among VRE; 56.6% isolates were accounting to *E. Faecium* followed by other enterococcus species (35.85%) followed by *E. Faecalis* (7.55%) in our study. In a study done Mathur S et al. to study anti-microbial resistance patterns among enterococci at Ajmer, Rajasthan revealed 63.20% of *E. Faecalis* followed by 34.40% of *E. Faecium* and 2.46% of other enterococcus species.<sup>9</sup> In a study done at tertiary care hospital, Assam revealed Most (51.7%) of the Enterococcus isolates were *E. faecium* followed by *E. faecalis* (16.6%) and *E. durans* (2%). Species was not determined in 29.7% of the isolates.<sup>10</sup> The results of various other studies are shown in [Table 4]. The difference is attributed to study settings, admission patterns and case presentations.

Our study recorded 52.83% of male and 47.17% of females were infected with VRE. In a CANWARD study 2007 to 2013 revealed 50% male and 50% female distribution with 1: 1 ratio of male: female with no gender predominance.<sup>11</sup> In a prospective longitudinal study done in SICU to understand VRE also revealed no gender predominance.<sup>12</sup> Similar to our study 55.06% of male and 44.94% of female were found out to be infected with little male predominance in a study done at ShriSathyaSai Medical College and Research Institute.<sup>5</sup>

Maximum number of enterococcus isolates were from Urine (65.97%), followed by blood culture (11.13%), swab (10.50%) and others. Similar pattern was observed in VRE isolates. Similar results were found in a study done at tertiary care hospital, Kolkata by Mukherjee K. et al., with 80% enterococcus isolates from urine followed by 16% from pus and 3.3% from blood.<sup>13</sup> In a study done by Jada S et al., reported highest (40.30%) urine isolates followed by pus and other body fluids (31.90%) and blood (18%).<sup>5</sup>

In present study; maximum enterococcal species were isolated from departments like Surgery (24.6%), Medicine (21.8%), and Paediatrics (17.9%) followed by others. Among VRE isolates Medical ICU (28.3%) tops the list

**Table 1:** Vancomycin resistance characteristics

S. No.	Result	Inhibition zone (mm)		
		Susceptible	Intermediate	Resistant
1	Vancomycin Disk	≥17	15-16	≤14 and 1 or any discernable growth within zone of inhibition
2	Vancomycin MIC	<4	8-16	>32

**Table 2:** Distribution of enterococcus and VRE

Parameter	Enterococcus		Vancomycin Resistant Enterococcus	
	Number (N= 476)	Percentage (%)	Number (N= 53)	Percentage (%)
Urine	314	65.97	26	49.06
Blood culture	53	11.13	17	32.08
Swab	50	10.50	3	5.66
Pleural fluid	12	2.52	1	1.89
Pus	10	2.10	0	0.00
Endotracheal tip	7	1.47	1	1.89
Foley's tip	7	1.47	2	3.77
Cup tip	5	1.05	1	1.89
High vaginal swab	3	0.63	1	1.89
Cerebrospinal fluid	3	0.63	1	1.89
Tissue	3	0.63	0	0.00
Ascetic fluid	3	0.63	0	0.00
Drain	2	0.42	0	0.00
Abscess	2	0.42	0	0.00
Ryle's tip	2	0.42	0	0.00
Surgery	117	24.6	5	9.43
Medicine	104	21.8	5	9.43
Paediatrics	85	17.9	10	18.87
Medicine-ICU	44	9.2	15	28.30
Obstetrics and Gynaecology	35	7.4	2	3.77
OPD	28	5.9	0	0.00
Tuberculosis and respiratory medicine	19	4.0	6	11.32
Neonatal-ICU	18	3.8	7	13.21
Orthopedics	12	2.5	1	1.89
Surgery ICU	7	1.5	0	0.00
Obstetrics ICU	5	1.1	2	3.77
ENT	2	0.4	0	0.00

**Table 3:** Antibiotic susceptibility pattern of VRE

S. No.	Antimicrobial agent	Percentage (%) of resistant strains of VRE		
		<i>E. faecium</i> (n=30)	<i>E. faecalis</i> (n= 4)	Other enterococcus(n=19)
1	Penicilin	100	100	100
2	Ampicilin	100	100	100
3	Levofloxacin	100	100	100
4	Rifampicin	96.66	100	100
5	Nitrofurantoin	71.42	66.66	70
6	Erythromycin	82.75	100	88.88
7	Teicoplanin	66.66	75	78.95
8	Tetracyclin	37.5	66.66	37.5
9	Linezolid	20	25	15.79
10	Fosfomycin	3.44	25	5.55

**Table 4:** Comparison of VRE isolates

Study	<i>E. faeium</i>	<i>E. faecalis</i>	Other
Present study	56.60%	7.55%	35.85%
Mathur S et al., <sup>9</sup>	34.40%	63.20%	2.46%
Bhuyan B et al., <sup>10</sup>	51.70%	16.60%	29.70%
Sreeja S et al., <sup>6</sup>	24.00%	76.00%	0.00%
Tripathi A et al., <sup>8</sup>	39.00%	61.00%	0.00%

followed by Paediatrics (18.87%) followed by others. Similarly CANWARD study done to understand VRE epidemiology in Canadian hospitals revealed 45% VRE isolates were from medical ward, followed by 32.5% from ICU set up followed by 12.5 from surgical wards.<sup>11</sup>

In our study; antibiogram reported 100% resistance against commonly used antimicrobial agents like Penicilin, Ampicilin and Levofloxacin across various species of VRE. Most sensitive antimicrobial agents against VRE were Linezolid and Fosfomycin. In contrast to our study; a study done in tertiary care hospital, Nigeria reported 100% resistance of VRE against Linezolid.<sup>14</sup> Similarly a study done in Kolkata reported 70% resistance against Ampicilin 100% sensitivity towards Linezolid.<sup>13</sup> Similar findings were recorded in other studies.<sup>15–18</sup> Bhuyan B et al. recorded 79.4% resistance against penicillin, 67.9% against Ampicilin, and 69.6% against Levofloxacin. In addition similar to our study 0% resistance was recorded against Linezolid.<sup>10</sup> Regional practice of antimicrobial prescription, isolation standards, and antibiogram screening practices are the major influencers.

## 5. Conclusion

Enterococci are deft ecological occupants with an exceptional versatile ability to advance and transmit antimicrobial resistant determinants. Vancomycin resistant enterococci (VRE) are both of medical and public health importance associated with serious multidrug resistant infections and persistent colonization. It renders various significant helpful alternatives including "most advanced molecules" incapable and represents a critical bottle

neck for clinical administration. Rational prescription of anti-microbial agent, targeted VRE surveillance and timely antibiogram in admitted cases is the need of hour.

## 6. Source of Funding

None.

## 7. Conflict of Interest

None.

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