

# Speciation and Antibiogram of Enterococci in a Tertiary Care Centre with Special Reference to VRE

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## Abstract

**Background:** *Enterococci* are the normal commensals of the oral cavity, gastrointestinal tract and vagina. They have emerged as serious nosocomial pathogens and its multidrug resistance as a cause for concern. This study is undertaken to isolate and speciate *Enterococci* from various clinical specimens by biochemical methods and to study the antibiogram. **Methodology:** A total of 100 isolates from the clinical specimens like urine, pus, blood and body fluids were processed in the department of Microbiology, Adichunchanagiri Institute of Medical Sciences, B.G. Nagara, for a period of one and half year. The isolates were speciated by using conventional tests and antibiotic susceptibility testing was done by Kirby Bauer disc diffusion method. Confirmation of vancomycin susceptibility was done by the Epsilon test (E test) **Results:** Out of 100 *Enterococcal* isolates, majority were from age group of 51-60 years (24%) and male (58%). Most common species isolated was *E.faecalis* (74%) followed by *E.faecium* (26%). All isolates were sensitive to Teicoplanin and Linezolid. Majority of the isolates were resistant to *Erythromycin* and *Ciprofloxacin*. 20 isolates were intermediately sensitive to *Vancomycin* by the Kirby Bauer disc diffusion method. All intermediately sensitive isolates to *Vancomycin* were further tested by the E test and they were found to be *Vancomycin* sensitive. **Conclusion:** There is an increase in the rate of infection and antibiotic resistance in the *Enterococcus* species. The emergence of *Vancomycin* resistant *Enterococci* (VRE) presents a serious challenge for clinicians treating the patients and the Kirby Bauer disc diffusion method is not an accurate method for detecting the VRE.

**Keywords:** *Enterococcus*; Speciation; VRE.

## Introduction

*Enterococci* are one of the emerging nosocomial infections. *Enterococci* can present as commensals forming the indigenous flora of intestinal tract, oral cavity and vagina [1]. The most frequent infections caused by them are urinary tract infections [UTIs] followed by wound infections, biliary tract infections, intra-abdominal infection, rarely septicaemia, meningitis, endocarditis, bacteraemia and pelvic infection [2].

Although many of *Enterococcus* species have been identified, only two namely *E.faecalis*, *E.faecium* are responsible for 95% of human infections caused

by *Enterococci*. The other common species include *E.durans*, *E.casseliflavus*, *E.avium*, *E.gallinarum*, *E.hirae*, *E.mundtii*, *E.malodoratus* and *E.salitorius*.

They exhibit both intrinsic and acquired resistance to aminoglycoside and cephalosporin. The emergence of vancomycin resistant *Enterococci* (VRE) in addition to the increasing incidence of high level aminoglycoside resistance (HLAR), presents a serious challenge for clinicians treating the patients with infections due to *Enterococci*. So it is important to monitor resistance pattern for *Enterococci* in hospital regularly [2-4]. The aim of our study was to determine the prevalence of *Enterococcus* from various clinical specimens and to

determine the antibiogram with special reference to the vancomycin susceptibility.

### Methodology

A total of 100 isolates from the clinical specimens like urine, pus, blood and body fluids from the patients attending AH & RC, B.G. Nagara, were processed in the department of Microbiology, Adichunchanagiri Institute of Medical Sciences, B.G. Nagara., for a period of one and half year. The specimens were inoculated onto Blood agar, MacConkey agar and were incubated overnight at 37°C. Presumptive identification was done by using standard protocol like gram staining, catalase test, bile esculin test, heat and salt tolerance test and  $\alpha$ -pyrrolidonyl  $\beta$ -naphthylamide (PYR) test [Figure 1 & 2]. Speciation was done using sugar fermentation test, arginine hydrolysis, growth in pyruvate broth, motility and pigment production [Figure 3 & 4] [2,5].

The antimicrobial susceptibility testing was performed by the Kirby Bauer disc diffusion method by using commercially available antimicrobial discs (Himedia®) like Ampicillin (10 $\mu$ g), vancomycin

(30 $\mu$ g), Erythromycin (15 $\mu$ g), Tetracycline (30 $\mu$ g), ciprofloxacin (5 $\mu$ g), High level gentamicin (120 $\mu$ g), teicoplanin (30 $\mu$ g) and linezolid (30 $\mu$ g). The results were interpreted as per the CLSI guidelines using *E. faecalis* ATCC29212 as the control strains. The MIC of *Vancomycin* was determined by the E test for all the *Enterococci* isolates which showed intermediate sensitivity by the Kirby Bauer disc diffusion method [Figure 5]. The zone of inhibition was observed in the form of an ellipse and interpreted as per the CLSI guidelines [6].



Fig. 1: Bile-esculin agar showing black colored *Enterococcus* colonies.

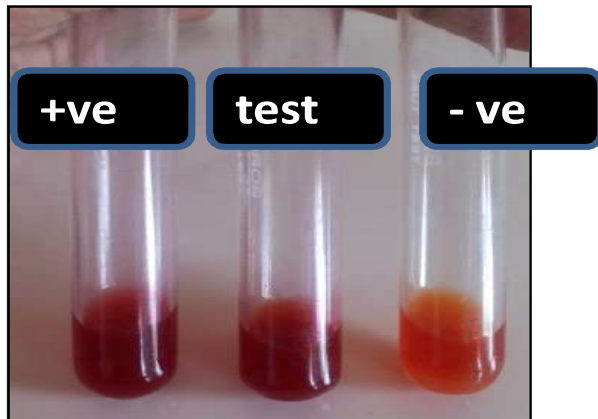


Fig. 2: PYR test.

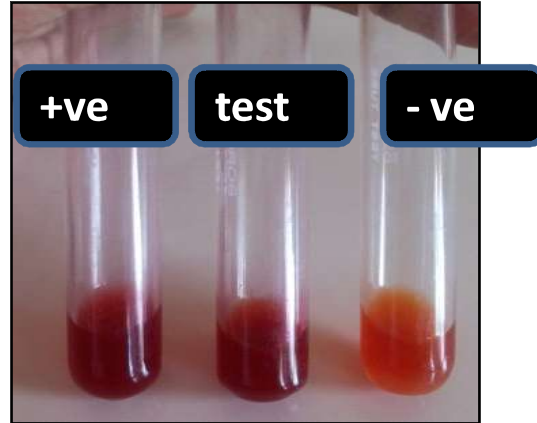


Fig. 3: Arginine hydrolysis by *E. faecalis*.

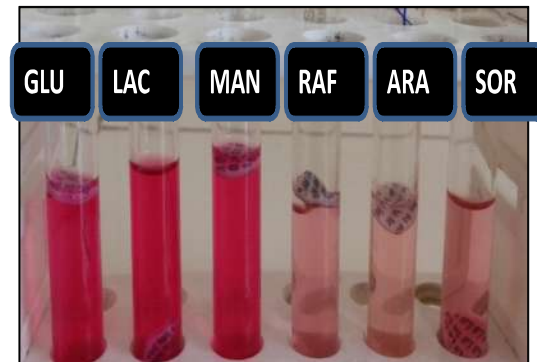


Fig. 4: Fermentation of sugars by *E. faecalis*.



Fig. 5: MIC for *Vancomycin* by E-test.

## Results

Out of 100 *Enterococcal* isolates, majority were in the age group of 51-60Years 24 (24%) and male 58 (58%). Age and gender wise distribution is shown in Table 1.

Majority of isolates were from urine sample (52%) and patients admitted in Medicine ward

(40%). The common species isolated was *E.faecalis* (74%) followed by *E.faecium* (26%). All *Enterococcal* isolates were sensitive to Teicoplanin and Linezolid and Antibiotic susceptibility pattern of *Enterococci* is shown in Table 2. 20% of isolates showed an intermediate sensitivity to *Vancomycin* by the Kirby Bauer disc diffusion method. These isolates were sensitive to vancomycin; with a MIC of less than 4µg which was determined by E test [Table 3].

**Table 1:** Age and gender wise distribution

Age in Years	Male (%) n=58	Female (%) n=42	Total (%) n=100
0-10	10 (17.2)	5 (11.9)	15 (15)
11-20	1 (1.7)	3 (7.14)	4 (4)
21-30	5 (8.6)	7 (16.6)	12 (12)
31-40	4 (6.9)	5 (11.9)	9 (9)
41-50	13 (22.4)	5 (11.9)	18 (18)
51-60	16 (27.5)	8 (19.04)	24 (24)
61-70	8 (13.8)	7 (16.6)	15 (15)
71-80	1 (1.7)	2 (4.8)	3 (3)
Total	58 (58)	42 (42)	100(100)

**Table 2:** Antibiotic susceptibility pattern of *Enterococcal* species

Antibiotics	<i>E.faecalis</i> (n=74)		<i>E.faecium</i> (n=26)	
	Sensitive n (%)	Resistance n (%)	Sensitive n (%)	Resistance n (%)
Ampicillin	60 (81)	14 (18.9)	7 (27)	19 (73)
Teicoplanin	74 (100)	0 (0)	26 (100)	0 (0)
Tetracycline	38 (51.4)	36 (48.6)	11 (42.3)	15 (57.7)
Erythromycin	24 (32.4)	50 (67.6)	2 (7.7)	24 (92.3)
Ciprofloxacin	25 (33.8)	49 (66.2)	3 (11.5)	23 (88.5)
Linezolid	74 (100)	0 (0)	26 (100)	0 (0)
High level gentamicin	60 (81)	14 (18.9)	7 (27)	19 (73)

**Table 3:** *Vancomycin* susceptibility testing

Sensitivity pattern	Kirby Bauer disc diffusion method (%)	E-test (%)
Sensitive	80	100
Intermediate Sensitive	20	0
Resistant	0	0

## Discussion

*Enterococci* are commensals of the gastrointestinal tract of human beings. Although a dozen of *Enterococcus* species have been identified, only two are responsible for the majority of human infections, i.e., *E.faecalis* and *E.faecium*. Over the past two decades they have become important nosocomial pathogens probably due to inherent resistance to antibiotics (cephalosporins), ability to

adhere to indwelling medical devices and ability to survive adverse environmental conditions [7]. The emergence of VRE is a cause of concern, since they are very difficult to treat and control. Correct speciation is very important since there is variation in resistance to antibiotics by particular *Enterococcal* species [8].

In the present study, the majority of *Enterococcal* isolates were from urine sample (52%) which is correlating with the study of Shinde RS et al. (53%).

*E.faecalis* (74%) is the predominant species isolated followed by *E.faecium* and which is comparable with the study of Desai PJ et al. (49.5%), Chakraborty A et al. (90.85%) and Toledo C et al. (82.6 %)[8-11].

Majority of *Enterococci* showed resistance to *Erythromycin* (74%) and *Ciprofloxacin* (72%), which is comparable with the study of Parameswarappa J et al [12]. High level gentamicin resistance was seen in 33% of isolates, which is comparable with Shinde RS et al. (44%) and Toledo C et al. (48.6%) [9,11]. 20% of the *Enterococcus*, which showed intermediate sensitivity to vancomycin by the Kirby Bauer disc diffusion method, were further tested by E test. The isolates were found to be sensitive to *Vancomycin* by E test.

*The inaccuracy of the disk diffusion method has resulted in an unwarranted utilization of this drug as a part of the treatment regimens. Therefore, a routine MIC monitoring of important antibiotics like vancomycin has to be done, before reporting it as resistant or intermediately sensitive*[13].

### Conclusion

The *Enterococcus* species have now emerged as nosocomial pathogens. Hence, it is important to know the changing patterns of the *Enterococcus* infections and the antimicrobial susceptibility patterns of the isolates. In addition to the increasing incidence, emergence of VRE presents a serious challenge for clinicians. Thus proper isolation, identification and knowing antibiotic susceptibility pattern will help in the early identification of resistant isolates and preventing their spread.

It is the role of the microbiologists to give prompt reports by using appropriate procedures in laboratories and to prevent the emergence of resistance by taking standard precautions and formulation of antibiotic policy in the institution for the proper use of antimicrobials by physicians [1].

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