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## Characterisation and Antimicrobial Susceptibility Pattern With Special Reference To Vancomycin Resistance In Various Clinical Isolates Of Enterococci.

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### ABSTRACT

Enterococci, gram positive bacteria which is known as a commensal bacterium in the gut has now gaining the attention and posing a challenge causing infections in the hospital milieu. This can be attributed to the ability of bacteria to exhibit both intrinsic and acquired resistance properties. The objective of the study is to isolate and speciation of Enterococci from various clinical specimens, to study the antibiogram of enterococcal isolates and to detect vancomycin resistance of Enterococci by Epsilometer method (E-test). Isolation and speciation of the isolates were done by conducting battery of biochemical tests as per standard protocols. Susceptibility of bacteria to various antibiotics was determined by Kirby Bauer disc diffusion method as per CLSI guidelines. MIC of vancomycin was detected using E-strip method. Overall, 104 strains of Enterococci were isolated from all the clinical samples except stool. Out of 104 strains, 63 (60.6%) were *E. faecalis*, 41(39.4%) were *E. faecium*. 100 percent sensitivity was seen to linezolid. 95.2% of the isolates were sensitive to vancomycin, 4.8% were intermediately sensitive by the Kirby Bauer disc diffusion method. All the isolates were further tested to detect minimum inhibitory concentration (MIC) of Vancomycin by the E test and they were found to be vancomycin sensitive (MIC<4mcg/ml). Enterococci causes variety of infections and it is intrinsically resistant to commonly used antibiotics. There is no good concordance between disc diffusion and E-test and hence routine MIC monitoring for antibiotics like vancomycin should be made mandatory.

**Keywords:** Enterococci, Vancomycin resistant Enterococci (VRE), Minimum inhibitory concentration (MIC), E-Test, Intermediate Sensitivity.

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## INTRODUCTION

Enterococci, Gram positive cocci, from the ages being considered as the coloniser of the gut, now showing the footprints as an established pathogen in hospitals. Well known as a ubiquitous bacterium commonly found in soil, water and food it is showing its pathogenicity in causing various diseases [1].

Enterococci commonly causes urinary tract infection followed by skin and soft tissue and blood stream infections [2, 3]. Among all the infections, urinary tract infection ranks second and ranks third among blood stream infections [4, 5]. However, there are few studies on incidence of infections by Enterococcus probably because of frequent misidentification and not reporting it as pathogen as it is a part of commensal flora [6]. Hence, proper identification and documentation of enterococci as a pathogen and to identify the is plays an important role in understand the spectrum of infections caused by commensals and provide treatment as well as to undertake infection prevention and control measures in any health care institution [7]. In a survey conducted by the Centre for Disease Control and Prevention (CDC), Enterococcus accounted for 13.9% of nosocomial infections [8]. This could be because of their antibiotic resistance mechanisms. Enterococci are intrinsically resistant to few antibiotics also, they have the ability to attain acquired resistance, there by developing resistance to commonly used antibiotics. Injudicious use of antibiotics and colonization pressure are the most common scenarios that lead to acquisition of drug resistance in Enterococci [9]. Blood stream infections due to vancomycin resistant Enterococci (VRE) poses a major challenge in patients undergoing gastrointestinal surgeries and is associated with mortality rates ranging from 33% to 68% [10]. Hence, it highlights the necessity of screening for Vancomycin resistance [11]. Hence, the present study was done aiming at isolation, identification, speciation of Enterococci, to determine antibiotic susceptibility pattern and vancomycin resistance from various clinical specimens. This study could throw light on the establishment of infections by commensal that help the clinicians for better management. To formulate and implement hospital antibiotic policy, to follow infection control protocols which can help in achieving comprehensive health care.

### Objectives

Isolation and identification of Enterococci from various specimens, to speciate the Enterococci by various phenotypic methods to determine the resistance of enterococcal isolates and to detect vancomycin resistance of Enterococci by Epsilometer (E-test).

## MATERIALS AND METHODS

The present study was carried out in the Department of Microbiology, Mysore Medical College and Research Institute, Mysore for a period of one year. The study has approved the ethical clearance and endorsement letter to continue the study has been provided by the institute. The consent from the patients was taken by the institution at the time of admission for all investigations and give the approval for the utilisation of the same for medical research purpose. All Enterococci isolated from various clinical samples such as pus, urine, blood, sputum, CSF and other body fluids from hospitals attached to Mysore Medical College and Research Institute, Mysore, for a period of one year were included in the study. The samples were processed for isolation, identification and speciation of Enterococci, antimicrobial susceptibility testing and detection of vancomycin resistance by using E strip following the standard protocols. All the samples except blood were inoculated onto Nutrient agar, Blood agar and MacConkey agar plates. All plates were incubated aerobically at 37°C and observed for growth at 24 and 48 hours. Blood collected in Brain Heart Infusion (BHI) broth was first incubated at 37°C for 24 hours. The bottles were examined for turbidity and subculture for 7 days at regular intervals [13-15]. Speciation is done by a series of biochemical tests like catalase test, bile esculin test, growth in the presence of 6.5% Sodium Chloride, growth at 45°C and 60°C, growth at high alkaline pH 9.6, fermentation of sugars, arginine hydrolysis, pyruvate utilization [16]. The Enterococci isolated were subjected to antibiotic susceptibility testing by Kirby - Bauer disc diffusion technique according to CLSI guidelines [17]. In the present study the susceptibility testing was carried out against the following antibiotics. Ampicillin (10mcg) High level gentamicin (120mcg) Ciprofloxacin (5mcg) Tetracycline (30mcg) Nitrofurantoin (300mcg) Tigecycline (15mcg) Erythromycin (15mcg) Chloramphenicol (30mcg) Vancomycin (30mcg) Linezolid (30mcg) MIC testing is done by E- strip method using Mueller Hinton Agar. The Epsilometer (E) test is an agar diffusion method which utilizes a predefined continuous and exponential gradient of antibiotic concentrations

immobilized along a rectangular strip. For testing vancomycin susceptibility, a strip with concentration gradient of 0.016 to 256 µg/ml was used [18-20].

**RESULTS**

During the 12-month study period, 104 isolates of Enterococci from patients of different age group, admitted in various medical, surgical wards, ICU in Hospitals attached to Mysore Medical College and Research Institute, Mysore, were included in the study.

Out of 104 isolates, maximum number of cases (n=41, 39.4%) were seen in the age group of 17-45yrs, followed by 46-60yrs (n=20,19.2%), 60-80yrs (n=15, 14.4%) and least number of cases were in the age group 1-28 days (n=7, 6.73%). The mean age is 34.21yrs. Out of 104 cases studied 94 cases (90.4%) were inpatient and 10(9.6%) were outpatients.

**Table 1: Number of enterococci isolated from various sample**

Sample	No. Of cases	Percentage
Urine	46	44.2
Pus	36	34.6
Blood	13	12.5
Sputum	8	7.7
Pleural fluid	1	1.0
Total	104	100

Chi-square=71.288; p=.000

Out of 104 isolates, Urine samples constitute majority of the specimens accounting for 44.2% (n=46) followed by pus samples 34.6%, (n=36), blood samples 12.5% (n=13), sputum 7.7% (n=8) and pleural fluid 1% (n=1).

*E. faecalis* and *E. faecium* are the two species that were isolated. Out of 104 isolates, *E. faecalis* were 63(60.6%) which is the most predominant species, followed by *E. faecium* 41(39.4%).

**Table 2: Antibiotic susceptibility pattern of Enterococci**

Antibiotics	Resistance		Sensitive		Moderately Sensitive N (%)	NA
	No. of cases	Percentage (%)	No of cases	Percentage (%)		
Ampicillin	90	87	14	13	----	----
Ciprofloxacin	71	68	33	32	----	----
HLG	27	26	77	74	----	----
Tetracycline	70	67	34	33	----	----
Nitrofurantoin	10	10	37	36	----	57
Chloramphenicol	13	13	91	88	----	----
Erythromycin	79	76	25	24	----	----
Tigecycline	25	24	79	76	----	----
Linezolid	0	0	104	100	----	----
Vancomycin	0	0	99	95	5(4.8%)	----

Of 104 isolates maximum resistance was seen to ampicillin 87%(n=90), followed by Erythromycin 76%(n=79), ciprofloxacin 68%(n=71), tetracycline 67%(n=70), high level gentamicin 26%, tigecycline 24%, chloramphenicol 13%, nitrofurantoin 10%. All the isolates were sensitive to linezolid, 95 % (n=99) cases showed vancomycin sensitive, 4.8 % (n=5) cases showed moderate sensitivity (15- 16mm zone size). Out of 41 isolates of *E. faecium*, 38 isolates were sensitive to vancomycin and 3 isolates showed moderate sensitivity. Among 63 *E. faecalis* isolates, 61 were sensitive and 2 were moderately sensitive. None of the isolates were resistant to vancomycin.

**Table 3: Vancomycin susceptibility comparison by disc diffusion and E. strip.**

Pattern	Disc diffusion method		E-strip method	
	No. of Cases	%	No. of Cases	%
Vancomycin sensitive	99	(95.2%)	104	(100%)
Vancomycin moderately sensitive	5	(4.8%)	0	(0%)
Total	104	(100%)	104	(100%)

Cramer’s V=.157; p=.024

Of 104 Enterococci isolated, 99(95.2%) were sensitive to vancomycin whereas 5(4.8%) were moderately sensitive by disc diffusion method. However when these isolates were tested with E.strip all the isolates (100%) were sensitive with the MIC less than 4mcg/ml.

### DISCUSSION

Enterococci, a commensal bacterium from decades, now emerged as organisms capable of causing grievous infections causing mortality in humans, more in the nosocomial environment. A state of being a commensal and pathogen a double role is facilitated by its intrinsic and acquired resistance to all antibiotics currently in use. With rising incidence of vancomycin resistance association with high mortality rates makes the bacteria a challenge. Increase in the infection rate VRE is posing a great challenge and concern to physicians and also to public health authorities. The overall antibiotic resistance and vancomycin resistance are found to vary greatly in different places and in different hospitals. In the present study, an attempt has been made to characterize and speciate Enterococci, to know their antibiotic susceptibility pattern and to detect vancomycin resistance by E-strip.

The present study showed maximum number of cases in the age group of 17- 45yrs, mean age being 34.2. Similar demographic findings were observed in a study done by Tripathi A et al [20] where in maximum number of cases were in the age group of 21-40yrs, and in a study done by Abdulhakim Abamecha et al [21] maximum number of cases were seen in the age group of 28-55yrs. However, in a study done by Sharma A et al [22] the most common age group was 0-20 yrs, and in another study by Bhatt P et al [23] it was >60yrs. In our study maximum number of patients were around 35 yrs of age. This could be due to the exposure of this group to various occupation based environmental conditions compared to other age groups. In our study 90.4% of the cases were in patients, who were admitted in various wards (Medical, surgical, obstetrics, paediatrics and various ICUs) and 9.6% were from the outpatient department. Similar distribution was observed in the studies done by Jyothi S et al [24], where in maximum cases were seen from in patient wards when compared to outpatient departments. The above studies provide a mirror image of Enterococci as a successful nosocomial pathogen. Irrational use of antibiotics, non-adherence to antibiotic policy, increase length of hospital stay, co-morbidities play an important role in acquiring the drug resistance [25-27].

In the present study maximum number of isolates were from the urine samples (44.2%), similar to the studies done by Sumangala et al [28], Kumar D et al [29], Getso et al [30], Yadav et al [31], Mukherjee et al [32], emphasizing on the fact that the urinary tract infection is the most common infection caused by enterococcus. Enterococci was reported as the third most frequent uropathogen in intensive care unit acquired urinary tract infections after Escherichia coli and Pseudomonas aeruginosa.

In the present study we isolated two species *E. faecalis* (60.6%), followed by *E. faecium* (39.4%). The results are similar to the studies done by Abamecha et al [21]. In our study *E. faecalis* was predominant species isolated followed by *E. faecium* which is similar to the studies done by Mwikuma G et al [34], Sachan S et al [4]. Both these species are long known to be significantly associated with the clinical disease hence their isolation is a cause of serious concern. Correct speciation plays a major role in the treatment since each species has different antibiotic resistance pattern. In the present study, ampicillin resistance was high in *E. faecium* (92.7%), followed by *E. faecalis* (82.5%), which is similar to other studies done by Adhikari L et al [35]. High recovery rate of ampicillin resistant Enterococci could be indicative of selective antibiotic pressure. A strategy for restricted antibiotic prescription may seem more appropriate and feasible in limiting the spread of these bacteria.

In the present study, the resistance to High level gentamicin was 27%, in that resistance in *E. faecalis* was more (28.6%), than in *E. faecium* 22%. However, in other studies by Das AK et al [36], where *E. faecium* showed more amino glycoside resistance than *E. faecalis*. In the present study, among the Enterococcus spp, *Enterococcus faecalis* showed more resistance than *Enterococcus faecium* to antibiotics like tetracycline, chloramphenicol, erythromycin, ciprofloxacin and Nitrofurantoin.

Knowledge on resistance of the organism helps in preparing an empirical therapy. In the present study, none of the isolates were resistant to vancomycin, similar to the studies done by Mwikuma G [33], in contrary Purohith et al [36] reported 22.8% of resistance. In the present study, vancomycin when tested by Kirby Bauer disc diffusion method, isolates were 95.1% (n=99) sensitive, 4.8% (n=5) moderately sensitive, and none of the isolates were resistant 0(0%). But, when the isolates were tested with E- test for the detection of MIC, all the isolates were sensitive with MIC < 4µg/ml. This error of detection between disc diffusion method and E- test is significant with p value of 0.02. Thus, this tells Kirby Bauer disc diffusion method in detecting the susceptibility to vancomycin is not an accurate one and detection of minimum inhibitory concentration is mandatory. The prevalence of VRE in India is much lower when compared to the western world. The emergence of VRE has been attributed to the injudicious use of vancomycin. colonization pressure is also an important variable that contributes for emergence of drug-resistant bacteria. Employment of disc diffusion method a method of choice for resistance detection which is an inaccurate method has resulted in an unwarranted utilization of this drug as a part of the treatment regimens. Therefore, a routine MIC monitoring of vancomycin has to be done, before reporting it as resistant or intermediately sensitive [37, 38].

### CONCLUSION

This study has shown that Enterococci can cause a diverse infection. In the present study, enterococcus showed resistance to various commonly used antibiotics. The study shows a discrepancy in the sensitivity of vancomycin among disc diffusion and E-strip method, the error was statistically significant with p=0.02. This shows that E. strip method is more reliable than disc diffusion test. Therefore, a routine MIC monitoring of important antibiotics like vancomycin has to be done, before reporting it as resistant or intermediately sensitive.

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