

## Speciation and Anti-Microbial Susceptibility Pattern of Enterococcal Isolates from Various Clinical Samples with Special Reference to Vancomycin Resistance

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### Abstract

**Background:** Enterococci are important causes of both communities acquired and nosocomial infections. They show intrinsic resistance to a number of commonly used antibiotics, particularly the cephalosporins. During the last few years, Enterococci have acquired resistance to a number of important antibiotics including glycopeptides. Enterococci resistant to all three antimicrobial agents (penicillin, aminoglycosides and Vancomycin) pose a serious challenge not only for clinicians but also for health care institutions. It results in treatment failure, selection and spreading of resistant strains in the health care institution. The increasing occurrence of Enterococcus species, worldwide, since late 1980s, is of particular concern due to the emergence of Vancomycin Resistant Enterococci (VRE). VRE has also been reported from some parts of India. The appearance of VRE has limited the therapeutic options available for clinicians. **Materials and Methods:** Study was carried out in the Department of Microbiology, Shivamogga Institute of Medical Sciences, Shivamogga, between November 2015 and October 2018. Enterococci were isolated from various clinical samples at a tertiary care hospital using the standard techniques. The isolated Enterococci are then tested for routine antibiotics sensitivity by disc diffusion method including Vancomycin sensitivity. **Results and Discussion:** A total 330 Enterococcus isolates were obtained from various clinical specimens such as C. Among 330 Enterococcus species, 235 species were Enterococcus faecalis and 95 species are Enterococcus faecium. The Enterococcal species showed 100% sensitivity to Vancomycin and Linezolid. The ability of the laboratory to identify enterococci and to detect Vancomycin resistance promptly and accurately is essential in recognizing VRE colonization and infection and avoiding complex, costly containment efforts that are required when recognition of the problem is delayed. Further, acquisition of Vancomycin resistance leaves few options for therapeutic management.

**Keywords:** Enterococcus Faecalis; Enterococcus Faecium; Vancomycin; Vre.

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### Introduction

Over the last two decades, Enterococci, known only as intestinal commensals with little significance have evolved as deadly pathogens. They are important causes of both communities acquired and nosocomial infections. They show

intrinsic resistance to a number of commonly used antibiotics, particularly the cephalosporins. During the last few years, enterococci have acquired resistance to a number of important antibiotics including glycopeptides. Enterococci resistant to all three antimicrobial agents (penicillin, aminoglycosides and vancomycin) pose a serious

challenge not only for clinicians but also for health care institutions. It results in treatment failure, selection and spreading of resistant strains in the health care institution [1].

The present study was aimed to speciate Enterococci which helps to know the prevalent species in and around Shimoga and also, detecting the antimicrobial resistance pattern among Enterococcus isolates obtained from various clinical specimen in a tertiary care hospital I with special emphasis on Vancomycin resistance.

## Materials and Methods

The present study was a cross sectional study carried out in the Department of Microbiology, Shimoga Institute of Medical Sciences, Shimoga, Karnataka, during November 2015 and October 2018. Various clinical samples like Urine, Pus, blood and body fluids from the patients attending McGann Teaching Hospital, Shimoga were used for the study. Specimen were collected in a sterile, proper labelled container with aseptic precautions and processed as per standard Microbiological procedures.

All specimens were screened for the pus cells and organism. Specimen was cultured on Blood agar and MacConkey and incubated for 37°C for 24 hours. Growth was then processed for Gram staining and catalase test. Gram positive cocci arranged in pairs which were catalase negative considered as streptococcus species. Enterococcus isolates were identified and speciation done by their colony morphology, Gram stain and various biochemical tests by standard conventional techniques.

### *Antibiotic susceptibility testing*

The antibiotic resistance profile was determined by Kirby-Bauer disc diffusion method using different antimicrobial agents supplied by manufacturer (HiMedia Laboratories, Mumbai) and interpreted according to guidelines recommended by Clinical and laboratory Standards Institute (CLSI) [5].

Susceptibility to Vancomycin was performed by Kirby-Bauer Disc Diffusion Method (KBDDM) on Mueller Hinton Agar by using 30µg Vancomycin disc (HiMedia) [2].

## Results

In the present study, a total of 330 Enterococcus were isolated from various clinical cases. Out of 330 cases, 227 (69%) patients were female and 103 (31%)

were male patients. The maximum percentage of isolation was seen among the age group 30-60 years. The sex distribution is shown in Table 1.

**Table 1:** Gender wise distribution of clinical samples used in the study

Sex	Number	Percent (%)
Male	227	69
Female	103	31
Total	330	100

Out of the total 330 various clinical samples, enterococcus isolated from urine (201), pus / exudates (69), blood (33) and, others (23). Majority of the enterococcus isolates were from urine, followed by pus and then blood (Table 2).

**Table 2:** Details of various clinical samples from which the enterococcus aureus was isolated.

Clinical Specimen	No. of Enterococcus isolates	Percentage
Urine Pus	201	62.1
Pus	69	20.9
Blood	33	11.8
Others (Body fluids)	23	5.1
Total	330	100

Maximum isolation of Enterococcus isolates was isolated from urine specimen. It indicates that urinary tract infections are the most common infections caused by Enterococci in our hospital.

Among 330 Enterococcus species isolated in our study, 235 species were Enterococcus faecalis and 95 species were Enterococcus faecium (Table 3). Most of the enterococcus isolates were resistant to routinely used antibiotics. All the isolates showed 100% sensitivity to Vancomycin and Linezolid.

**Table 3:** Species wise distribution of Enterococcus species

Enterococcus spp.	No. of isolates	Percentage
Enterococcus faecalis	235	71.2
Enterococcus faecium	95	28.8
Total	330	100

The percentage of antibiotic sensitivity of Enterococcus species to various antibiotics were differed (Table 4). Enterococcus species showed sensitivity of 35.5% to Ampicillin, 56% to Ciprofloxacin, Norfloxacin (26.4%), Nitrofurantoin (41.3%) and they showed 100% sensitivity for Vancomycin and Linezolid.

**Table 4:** Percentage of antibiotic sensitivity of Enterococcus species to other antibiotics

Antibiotic	Sensitivity	Resistance
Ampicillin (10µg)	117 (35.5%)	213 (64.5%)
Ciprofloxacin (5µg)	185 (56%)	145 (44%)

Norfloxacin*	53 (26.4%)	148 (73.6%)
Nitrofurantoin(30µg) *	83 (41.3%)	118 (58.7%)
Vancomycin (30µg)	330 (100%)	00
Linezolid (30µg)	330 (100%)	00

\*Antibiotic used for urine samples only (201 samples)

## Discussion

Enterococci are important causes of both communities acquired and nosocomial infections. They show intrinsic resistance to a number of commonly used antibiotics. During the last few years, enterococci have acquired resistance to a number of important antibiotics including glycopeptides. The increasing occurrence of enterococcus species, worldwide, since late 1980s, is of particular concern due to the emergence of Vancomycin Resistant Enterococci (VRE). VRE has also been reported from some parts of India. The appearance of VRE has limited the therapeutic options available for clinicians. Imprudent use of antibiotics and colonization pressure are the important causes of the drug resistance in Enterococci. In the present study, 330 Enterococcus isolates from various clinical specimens were used. Out of which maximum number of Enterococci were isolated from urine (62.1%) followed by Pus (21%) and blood. This is slightly lower than Ruoff et al., who isolated maximum number of Enterococci from urine (68.2%). In another study conducted by Talebi et al., maximum number of Enterococci were isolated from urine (85%) followed by Pus (15.5%). Antibiotic resistance among Enterococci is a challenging global problem. Antibiotic resistance seen among Enterococcal isolates may be intrinsic or acquired. In our study, the maximum resistance was observed against Ampicillin (64.3%). In another study carried out by Salem Bekhit et al., (2012) also reported high resistance of Ampicillin accounting for 70.4% resistance among the isolates. Our study has shown ciprofloxacin resistance of 44%, where the study of Sarika Jain et al., (2011) also reported high resistance of ciprofloxacin (75%). In the present study, the highest sensitivity (100%) was shown with Vancomycin and Linezolid among all samples.

## Conclusion

In vitro testing of antimicrobial susceptibility of all clinical enterococcal isolates, suitable modification of the usual susceptibility testing procedures, judicious use of antibiotics, systematic

surveillance and control of fecalcolonization of resistant enterococci in hospital staff are some of the measures to be adopted for control of the drug resistance in enterococci. The ability of the laboratory to identify Enterococci and to detect Vancomycin resistance promptly and accurately is essential in recognizing VRE colonization and infection and avoiding complex, costly containment efforts that are required when recognition of the problem is delayed.

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