

# Prevalence and Antibiotic Resistance Pattern of Clinical Isolates of Enterococci from Blood Samples in A Tertiary Care Hospital, Jamnagar, Gujarat, India

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## ABSTRACT

**Introduction:** In recent years, enterococci have become important nosocomial pathogens. Indiscriminate use of antibiotics, prolonged hospital stay, severity of illness and immunosuppression are responsible for nosocomial acquisition of drug resistant enterococci.

**Aim:** To determine the prevalence and anti-microbial resistance pattern of enterococci in blood samples.

**Materials and Methods:** This retrospective study was carried out in the Department of Microbiology, Shri M. P. Shah Government Medical College, Jamnagar, Gujarat from January 2014 to June 2014. A total of 540 blood samples were tested. The isolates were identified by Gram staining, colony characteristics, catalase test, black color on bile esculin agar and tolerance to 6.5% NaCl. Antibiotic susceptibility test was done by Kirby-Bauer disc diffusion method for Penicillin

(10 units), Azithromycin (30 mcg), Gentamicin (10 mcg), Ciprofloxacin (5 mcg), Tetracycline (30 mcg), Linezolid (30 mcg) and Vancomycin (30 mcg).

**Results:** The prevalence rate of enterococci in blood samples was 6.48%. Antibiotic susceptibility tests showed high level resistance to commonly used antibiotics like Penicillin (100%), Azithromycin (85.71%), Gentamicin (100%), Ciprofloxacin (82.85%) & Tetracycline (77.14%) and spread to newer drugs like Linezolid (5.71%) and Vancomycin (8.57%).

**Conclusion:** We conclude that enterococcal strains with high level of resistance to penicillin aminoglycosides and fluoroquinolones group of drugs. So there is urgent need for more rational and restricted use of antimicrobials and continuous monitoring, in order to minimize the selection and to prevent hospital spread of such strains.

**Keywords:** Antimicrobial resistance, Blood stream infections, Vancomycin.

## INTRODUCTION

Blood stream infections are an important cause of mortality and morbidity and they are among the most common health care associated infections [1]. Diseases which are associated with blood stream infections can be self limited infections or it may lead to life threatening sepsis, it needs aggressive and prompt antimicrobial therapy [2].

Bacteraemia and meningitis may occur in neonates and in adults it may lead to endocarditis. In both the sex, enterococcal infection is equally distributed [3]. Vancomycin resistant Enterococci (VRE) sepsis is emerging as a significant problem in the intensive care setting [4].

The treatment of multidrug resistance is a big challenge for the clinicians. Resistance to beta lactam and glycopeptides is now a common feature of many hospital isolates of enterococci, and resistance to linezolid and aminoglycosides also leads to problem [5].

Risk factors like indiscriminate use of antibiotics, prolonged

hospital stay, severity of illness and immunosuppression are responsible for nosocomial acquisition of drug resistant enterococci ultimately leading to environmental contamination and cross infections [6,7]. Resistance mechanism to enterococci can be intrinsic, that is naturally occurring antibiotic resistance. It can be rapidly acquired by plasmids [8], gene transfer [9] and/or by mutation [10].

The increasing resistance to antibacterial agents such as penicillins, aminoglycosides, and also to glycopeptides such as vancomycin, created an increasingly worrisome problem in clinical practice [11-13]. Present study was carried out for the determination of the enterococcal blood stream infections, its prevalence and antibiotic resistance pattern.

## MATERIALS AND METHODS

This retrospective study was carried out at Department of Microbiology M. P. Shah Government Medical College and GG Hospital, Jamnagar, Gujarat, India, from January 2014 to June 2014. Fresh blood sample were collected and added

immediately to sterile glucose broth aseptically. We have included blood samples and excluded other samples like urine, pus, tissue, stool and body fluids in the study. A total of 540 blood samples were received from the Pediatric and Medicine wards of the GG Hospital, Jamnagar.

Blood culture bottles were incubated overnight at 37°C in incubator. After incubation the next day samples were cultured on primary isolation media MacConkey agar, blood agar media and nutrient agar for biochemical test and the next day, culture was done on selective medium Bile Esculin agar. Colony characteristics on MacConkey agar medium was pin-point pink lactose fermenting colonies and colony characteristic on blood agar was non hemolytic and approx. size of 0.5-1mm. Enterococcal isolates were identified by using standard tests like examining the colony morphology, gram staining, the catalase test, black color on bile esculin agar and the salt tolerance test to 6.5% NaCl. On Hanging Drop preparation, they were non-motile. Antibiotic sensitivity test was done for the positive samples for enterococci, according to the CLSI(Clinical and Laboratory Standards Institute) guidelines [14] for penicillin (10 units), azithromycin (30mcg), Ciprofloxacin (5mcg), Tetracycline (30mcg), Linezolid (30mcg) and Vancomycin (30mcg) by the Kirby-Bauer disc diffusion method using Mueller Hinton (MH) agar plates [14]. ATCC *Enterococcus faecalis* 29212 was used as a control strain.

**Ethical Clearance:** it is a retrospective analysis of samples tested for routine laboratory diagnosis; hence ethical clearance is not necessary.

## STATISTICS ANALYSIS

Data was entered and analyzed in MS excel 2007.

## RESULTS

Out of 540 blood samples, 35(6.48%) of blood samples were positive for enterococci and 505(93.52%) were negative for enterococci. So the Prevalence rate for enterococci was 6.48%. [Table/Fig-1] shows positivity rate for enterococci spp. Species identification was not done for the enterococci.

Sample	n (%)
Positive for enterococci	35 (6.48%)
Negative for enterococci	505 (93.52%)

**[Table/Fig-1]:** Distribution samples according to positive and negative for enterococci (n=540).

Age and sex wise distribution of patients was done, [Table/Fig-2]. The maximum numbers of isolates 57.14% were seen in the 0-20 years age group followed by 22.85% in the 21-40 years age group. More numbers of enterococci were isolated from males (60%) than from females (40%).

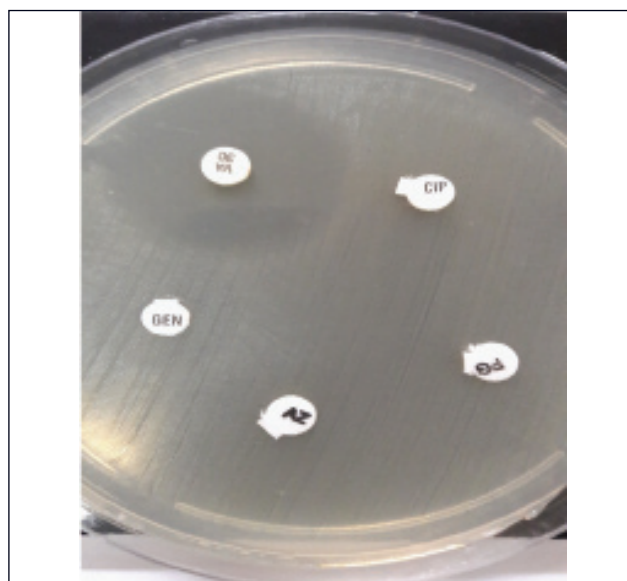
[Table/Fig-3,4] shows the resistance pattern of the enterococci. Resistance to penicillin and gentamicin was

Age group (yrs.)	Sex		Total
	Male	Female	
0-20	13 (37.14%)	7(20%)	20 (57.14%)
21-40	5(14.28%)	3(8.57%)	8(22.85%)
41-60	1(2.85%)	3(8.57%)	4(11.42%)
>61	2(5.71%)	1(2.85%)	3(8.57%)
Total	21(60%)	14(40%)	35(100%)

**[Table/Fig-2]:** Age and sex wise distribution of cases.

Antibiotic	Resistance No. (%)
Penicillin	35 (100%)
Azithromycin	30 (85.71%)
Gentamicin	35 (100%)
Ciprofloxacin	29 (82.85%)
Tetracycline	27 (77.14%)
Linezolid	2 (5.71%)
Vancomycin	3 (8.57%)

**[Table/Fig-3]:** Antibiotic resistant pattern of enterococci.



**[Table/Fig-4]:** Antibiotic Susceptibility test.

100% for the enterococci. Resistance to Azithromycin, Ciprofloxacin and Tetracycline was 85.71%, 82.85% and 77.14% respectively. Resistance also starts to develop to some higher antibiotics like Vancomycin & Linezolid that are 8.57% and 5.71% respectively.

## DISCUSSION

In the present study, the prevalence of *Enterococcus* isolates from the blood samples was 6.48% as shown in table-1. Study of Olawale KO et al., shows the prevalence rate was 5.9%, which is almost similar to present study [15].

Present study shows that highest number of enterococci about 57.14% were isolated from 0-20 years age group followed by 22.85% in 21-40 years age group as shown in [Table/Fig-2]. Almost similar result has been noticed in study of Anjana Telkar et al., [16] with more involvement of pediatric patients or teenagers with 54% cases followed by adult group with 30% cases. Study shows that enterococci were more common in pediatric patients or teenagers and could be associated with central nervous system infection while adult group could be associated with endocarditis [3].

As shown in [Table/Fig-2], 60% of males were affected to enterococcal blood stream infections as compared to 40% of females. Similar study was done by Anjana Telkar et al., it shows that 64% of males were affected to enterococcal blood stream infections as compared to 36% of females [16]. The reason for more enterococcal blood stream infections in male might be due to more exposure by male patients to environmental conditions that may lead to enterococcal infection. It might be due to some unexplained reasons. However, clinically there is no correlation between sex and *Enterococcus* infection and almost both sexes are equally affected [3].

Comparative study of Anjana Telkar et al., shows that, there was multidrug resistance to enterococci isolates and it shows that more than 50% resistance was seen to most of the drugs. In that study resistance to Linezolid and Vancomycin were 2.8% and 12% respectively [16]. Other study done by S. Sreeja et al., shows that resistance to Penicillin and Ciprofloxacin were 47% and 50% respectively [17]. Other recent studies had been done in the tertiary care hospitals in India for vancomycin resistance and showed that it may range between 1.7-20% [18-21].

The result of disc diffusion method can be inaccurate and can lead to utilization of this drug for treatment. So for the important antibiotics like vancomycin, MIC monitoring should be done before reporting as intermediate sensitive or resistant [22]. The imprudent use of vancomycin and non-compliance with the infection control measures lead to emergence of VRE (Vancomycin resistant enterococci) [21].

## CONCLUSION

There is high level of resistance to penicillin, fluoroquinolones and aminoglycosides group of drugs. So there is urgent need for more rational and restricted use of antimicrobials, in order to minimize the selection and to prevent such strains that spread in the hospital premises.

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