



## DISTRIBUTION AND IDENTIFICATION OF VARIOUS SPECIES OF ENTEROCOCCI FROM DIFFERENT CLINICAL SAMPLES WITH SPECIAL REFERENCE TO PHENOTYPIC CHARACTERIZATION & DRUG RESISTANCE IN A TERTIARY CARE CENTRE, GREATER NOIDA

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### ABSTRACT

**Background & objectives:** Enterococci are a part of normal intestinal flora of humans and animals but are increasingly being recognized as important human pathogens. Classified as group D streptococci, Enterococci are the second leading cause of nosocomial infections. The incidence of enterococcal infections and species prevalent in India is not thoroughly investigated. Although they cause only a few clinical infections in human beings, since the beginning of antibiotic era have posed major therapeutic challenges to humans. **Material & methods:** Various samples brought to Microbiology division of central laboratory such as blood, pus, urine etc. were included in the study. All the essential biochemical reactions required for identification upto the species level were done by conventional biochemical tests to establish the identity of Enterococci spp., followed by antibiotic sensitivity testing by Kirby Bauer disc diffusion technique. **Results:** In our study we found the higher isolation of *E. faecium* (53%) than *E. faecalis* (33%), maximum number of isolates were from females (73.43%) as compared to males (26.56%) and highest isolation from urine samples (69.09%) with Predominance in the sexually active age group (21-40yrs). **Discussion:** The present study was undertaken for phenotypic characterization of Enterococcus spp. with their Antibiotic resistance pattern. The highest isolation was from Obstetrics & Gynecology ward (74.42%) followed by Surgery ward (14.28%), probably due to catheterization, surgical procedures and prolonged hospitalization.

### KEYWORDS : Disk diffusion - enterococci - MIC - phenotype

#### INTRODUCTION

Enterococci, formerly classified with Streptococci, have been recognized to be of fecal origin since the beginning of this century. The genus Enterococcus comprises Gram positive cocci that are catalase negative, usually facultative anaerobic bacteria that grow in 6.5% NaCl, 40% bile salts, 0.1% methylene blue milk and at pH 9.6. They grow at 10°C and 45°C and can resist 30 min at 60°C(1-4). Over the past two decades, Enterococci have been identified as the agents of nosocomial infection with increasing frequency (5, 6). These organisms have survived in the hospital environment due to their intrinsic resistance to several commonly used antibiotics and more importantly their ability to acquire resistance to all currently available antibiotics, either by mutation or by receipt of foreign genetic material through the transfer of plasmids and transposons (7, 8, 9). Vancomycin has been used as the drug of choice in many resistant strains of Gram positive bacterial infections, especially those caused by Enterococci. There has been an increase in number of Vancomycin Resistant Enterococci [VRE] in recent times. The organism can also horizontally transfer this resistant determinant to other Vancomycin-susceptible species (10, 11). Resistance to Vancomycin is encoded by different clusters of genes referred to as the Vancomycin resistance gene clusters (e.g. VanA, VanB and VanC). Both high and low level Vancomycin resistance can occur in Enterococci. Low level Vancomycin resistance (VanC) is limited to 2 relatively non-virulent species: *E. gallinarum* and *E. casseliflavus*.

High-level resistance (encoded by VanA and VanB genes) is related to *E. faecalis* and *E. faecium*. The 4th Vancomycin resistance genotype, VanD described in strain of *E. faecium* exhibit moderate level of resistant to Vancomycin and Teicoplanin (8). Resistance to Linezolid is slowly developing, posing several questions on the virulence factors and their survival mechanisms (12). The Center for Disease Control and Prevention, in a survey on nosocomial infections, indicated that Enterococcus accounted for 13.9% infections, being next to *Escherichia coli* as a causative agent of hospital acquired urinary tract infections (13). Therefore, the same importance is given to the multidrug resistant Enterococcus species, like that of Methicillin Resistant *Staphylococcus Aureus* (MRSA) and Extended Spectrum Beta Lactamase (ESBL) producers, as nosocomial pathogens (14). Enterococci are the second leading cause of nosocomial infections (15), and VRE have been reported from worldwide(16-20).

The incidence of enterococcal infections and species prevalent in India is not thoroughly investigated. Few studies from India reported *E. faecalis* as the most prevalent species (1,9), with high-level resistance to aminoglycosides, but no resistance to vancomycin(21,22).

Based on the above facts this study was conducted with the objectives to find out the predominant species of Enterococcus in our hospital settings, to find out the extent of Multi-drug resistance pattern of the isolates and to create a baseline to keep a check on emergence of VRE for future studies and for hospital infection control purpose.

#### MATERIALS AND METHODS:

The study was conducted in the Department Of Microbiology, Noida International Institute of Medical Sciences & Hospital, Greater Noida, for a period of 1 year (August 2021 – Aug 2022). All patients irrespective of age and sex, who visited OPD or were admitted in different wards in NIIMS Hospital were included in the study. Various clinical samples like urine, blood, pus, sputum, endotracheal secretions, central venous line tips etc were collected & sent to bacteriology laboratory for culture and sensitivity with full precautions regarding sterility, from various wards and OPDs. specimens were processed according to standard bacteriological procedures available.

Preliminary identification of bacteria was based on colony characteristic of the organism i.e. colonial morphology, haemolysis on blood agar, changes in the physical appearance of the differential media and enzyme activity of the organism. The genus enterococcus was confirmed by Gram stain, esculin hydrolysis, hydrolysis of L-pyrrolidonyl-b-naphthylamide (PYR) test and salt tolerance (15,18). Speciation was based on Facklam's conventional method (23): tolerance to tellurite for *E. faecalis*, fermentation of arabinose, mannitol, raffinose and sorbitol for *E. faecium*(1,2,7,8) Antimicrobial susceptibility to penicillin, gentamicin, vancomycin, teicoplanin, ciprofloxacin and nitrofurantoin was determined by Kirby Bauer disk diffusion 16.

#### OBSERVATIONS & RESULTS

This study covers up the findings related to the type & frequency of clinical specimens during the study period of 1 year, drug resistance pattern with special reference to Vancomycin resistant Enterococci, species determination and Minimum Inhibitory Concentration (MIC) of Vancomycin against Enterococci. A total of 5401 samples were received for bacterial culture during the study period of 1 year. For further description in the study, all the samples were grouped under 5 heads- Urine, Pus, Blood, Body fluids (includes CSF, Pleural fluid etc) and Miscellaneous

**Table 5.1 Quantitative distribution of various samples**

Sample	Urine	Blood	Pus	Miscellaneous	Body fluids	Total
No. of sample	3732	630	490	426	123	5401
Percentage	69.09%	11.66%	9.07%	7.88%	2.27%	100%

Out of 64 isolates, 73.43% were from female patients and 26.56% were from male patients indicating higher rate of Enterococcal infection among females than the infection in males.

**Table 5.2 Gender distribution of various samples**

Females n (%)	Males n (%)
47 (73.43)	17 (26.56)

The maximum numbers of isolates were obtained from patients of the age group 21-40 years (62.5%) and no isolate was obtained from patients above the age of 60 years.

This signifies that young adults are more prone to Enterococcal infections.

**Table 5.3 Age wise distribution of samples**

Age Group (years)	No. of samples	Percentage
0-20	9	14.06%
21-40	40	62.5%
41-60	15	23.43%
>60	0	0%

Among all received samples, the samples received from patients were more from IPD (76.56%) than OPD (23.43%) Among the IPD cases, highest isolation was from Obstetrics & Gynecology ward (74.42%) followed by Surgery ward (14.28%).

**Table 5.4 Number of samples from IPD and OPD**

IPD	OPD
49	15
76.56%	23.43%

**Table 5.5 Distribution of samples in different wards**

Ward	Obstetrics & Gynaecology	Paediatrics	Medicine	Surgery	TB Chest
Samples	35 (71.42%)	3 (6.12%)	3 (6.12%)	7 (14.28%)	1 (2.04%)

On the basis of biochemical identification, speciation of 64 Enterococcal isolates were done and it revealed 95.31% E.faecalis and 4.68% E.faecium.

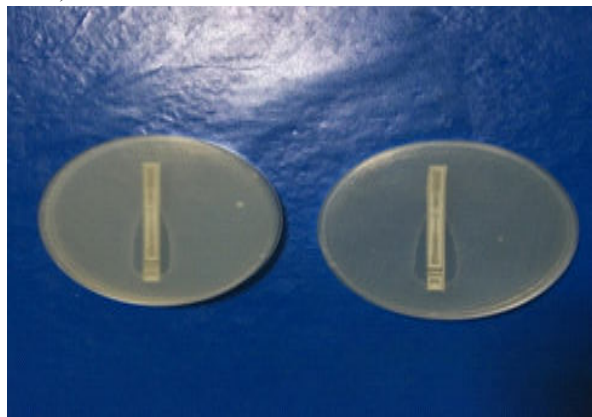
**Table 5.6 Isolation rate of different Enterococcus spp**

S.No.	Genospecies	Total number (n=64)	Percentage
1.	E.faecalis	61	95.31%
2.	E.faecium	3	4.68%

**ANTIBIOTIC SUSCEPTIBILITY PATTERN OF ENTEROCOCCI**

There were 7 classes of antibiotics tested for antibiotic susceptibility in all isolates (n=64) which includes Penicillins (Penicillin), Glycopeptides (Vancomycin, Teicoplanin), Tetracyclines (Tetracycline), Fluoroquinolones (Ciprofloxacin, Levofloxacin, Norfloxacin), Nitrofurantoin (Nitrofurantoin), Oxazolidinones (Linezolid), Aminoglycosides (Gentamycin, Streptomycin), Norfloxacin & Nitrofurantoin were used for urine isolates (n=50).

Minimum Inhibitory Concentration (MIC) of Vancomycin was tested by E-test method and revealed 100% sensitive results (Fig. given below)



**Table 5.7 Antibiotic Susceptibility pattern of Enterococcus**

	P		TEI		TE		CIP		LE	
	R	S	R	S	R	S	R	S	R	S
E. faecalis (%)	36	25	0	6	30	31	39	22	31	30
	59%	40.9%	0%	9.8%	49.1%	50.8%	63.9%	36%	50.8%	36%
E. faecium (%)	2	1	0	3	2	1	2	1	3	0
	66.6%	33.3%	0%	100%	66.6%	33.3%	66.6%	33.3%	100%	0%
Total (%)	38	26	0	64	32	32	41	23	34	30
	59.3%	40.6%	0%	100%	50%	50%	64%	35.9%	53.1%	42.8%

	NX		NIT		LZ		HLG		HLS	
	R	S	R	S	R	S	R	S	R	S
E. faecalis (%)	38	10	6	42	0	61	26	35	25	36
	79.1%	16.3%	15.7%	68.8%	0%	100%	42.6%	57.3%	40.9%	59%
E. faecium (%)	1	1	0	2	0	3	2	1	1	2
	33.3%	33.3%	0%	66.6%	0%	100%	66.6%	3.3%	33.3%	66.6%
Total	39	11	6	44	0	64	28	36	26	38

It was observed that Vancomycin, Teicoplanin and Linezolid were 100% sensitive against Enterococcus faecalis and Enterococcus faecium whereas the total highest resistance was seen against Ciprofloxacin 64.06% followed by Penicillin (59.37%).

**DISCUSSION**

This study investigated the conventional method of isolation, identification, and speciation. The series of tests employed in this study identified all the isolates to the species level. Conventional test scheme proposed by Facklam & Collins were thus successfully used for the speciation of Enterococci. Enterococci were isolated from various clinical specimens with prevalence rate of 4.2% which was similar to study done by Mukherjee et al., 2016 (4.8%) . While other study conducted by Desai PJ et al., 2001 showed higher prevalence (22.19%) and S Sreeja et al., 2012 showed low prevalence (2.3%)

The present study showed maximum number of isolates were from females (73.43%) as compared to males (26.56%). A similar result was shown in a study conducted by P. Srivastava et al., 2013 showing more number of isolates from females (72%) than from males. (28%) On the contrary, the study done by Ali, Shamshad et al., 2014 showed higher number of isolates from males (66%) than females (34%). A study conducted by Nautiyal S et al., 2016 also showed higher number of isolates from males (55.50%) than females (44.50%).

The high prevalence of Enterococcal infection in this study was seen in age group of 21-40 years (62.5%) which is in accordance with other studies done by P. Srivastava et al., 2013 (40%) . E. faecalis (95.31%) and E. faecium (4.68%) were the common species isolated from various clinical samples. Similar results were shown in the study of Trupti B. Naik et al 2016., with higher isolation of E. faecalis (84.84%) than E. faecium (15.15%). While study done by Jain S et al., 2011 showed higher isolation of E. faecium (53%) than E. faecalis (33%). Speciation of Enterococcus is important as they are known to be associated with the long term hospital acquired infections.

The present study demonstrated maximum ciprofloxacin resistance (64.06%) which is similar to study done by Latika et al 2012 (62%) and higher than a study done by Trupti B. Naik et al 2016 (37.5%) . Similarly, in the present study Norfloxacin resistance was found to be 78% which is higher than the study done by Trupti B Naik et al., 2016 (43.75%).

Penicillin along with aminoglycosides is a drug of choice to treat many Enterococcal infections, therefore Enterococcal resistance against these antibiotics has an important clinical implication. In the present study, the second most common antibiotic resistant was seen against Penicillin (59.37%) whereas study conducted by S. Sreeja et al., 2012, showed low resistant (47%). The high rate of resistance to penicillin may be due to production of β-lactamases or low affinity penicillin binding proteins. Hence, frequent use of penicillin and quinolones for the empirical treatment of endemic infectious diseases may result in

high proportion of antibiotic resistant species seen in the isolates.

Pattern of High Level Aminoglycoside Resistance is important because it has its role in synergistic effect with cell wall synthesis inhibitors like Vancomycin and penicillin. High Level Gentamicin Resistance was reported to be 66.66% and High Level Streptomycin Resistance was reported to be 33.33%. Similar results were seen in study conducted by Narayan Shrihari et al., 2011 showing 43.14% HLGR and 41.17% HLSR in *E.faecalis* and 66.67% HLGR and 33.33% HLSR in *E.faecium*.

The high prevalence of multidrug resistant Enterococcal infection in a tertiary care set up is due to excessive and indiscriminate use of broad spectrum antibiotics and high rate of patient transfer from Peripheral Centres.

## CONCLUSION

Due to the increasing scenario of drug resistance and emergence of various species of Enterococcus, there is a need to carry out regular surveillance of antimicrobial resistance pattern of Enterococci so as to provide proper therapy and to prevent the spread of resistant isolates. Proper diagnosis and efficient infection control measures can restrict the spread of infection. In conclusion, more advanced and sensitive molecular methods are to be employed to determine the common clones/strains of hospital and community acquired Enterococci in order to control or at least delay the future emergence of Enterococcus spp. as potential pathogen

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