

# Characterisation and Antibiotic Susceptibility Pattern of Enterococci in a Tertiary Care Hospital of North East India

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## ABSTRACT

**Introduction:** Enterococci are important agents of nosocomial infection, ranking as the second most common organisms causing complicated urinary tract infections, bacteraemia, endocarditis, intra-abdominal and pelvic infections, wound and soft tissue infections, neonatal sepsis and rarely meningitis. Infections by enterococci have traditionally been treated with cell wall active agents (e.g., penicillin or ampicillin) in combination with an aminoglycoside (streptomycin/gentamicin); however, emergence of High Level Aminoglycoside Resistance (HLAR), beta-lactam antibiotics resistance and vancomycin resistance by some strains has led to failure of synergistic effects of combination therapy.

**Aim:** To characterise enterococci up to the species level and study their antibiotic susceptibility pattern.

**Materials and Methods:** The present study was a cross-sectional study in which a total of 14114 clinical specimens, obtained during the period from September 2018 to August 2020 in this cross-sectional study, were tested to identify and speciate enterococcal isolates using standard microbiological

methodology. Antibiotic susceptibility testing was done by Kirby Bauer disc diffusion method. Data was analysed using descriptive statistics (percentage and proportion).

**Results:** During the study period of two years, 146 enterococci were recovered from 14114 different clinical samples, accounting for an infection rate of 1.03%. Among 146 enterococcal isolates, 116 (79.5%) were obtained from urine, 13 (8.9%) from blood, 10 (6.8%) from pus, 4 (2.7%) from wound swab and 3 (2.1%) from catheter tip. The predominant isolates were *E. faecalis* (82.2%) followed by *E. faecium* (15.8%), *E. durans* (1.3%) and *E. gallinarum* (0.7%). On studying the antibiotic susceptibility pattern, most of enterococcal isolates were predominantly resistant to ampicillin and ciprofloxacin (73.9% in both) and least resistant to linezolid (3.4%).

**Conclusion:** *Enterococcus faecalis* and *Enterococcus faecium* were the predominant species in present study and majority of the isolates was sensitive to linezolid (96.6%). Therefore, it is necessary to implement infection control measures like antimicrobial stewardship especially restricting the use of antibiotics to minimum.

**Keywords:** Antibiogram, Nosocomial, Speciation

## INTRODUCTION

Enterococci are gram positive cocci that occur mainly in pairs but also in short chains and produce smooth, gray colonies that are either non-haemolytic or alpha haemolytic [1,2]. They were distinguished from streptococci and related taxonomy by their ability to grow at 10°C and 45°C, and growth in the presence of pyrrolidonylarylamidase [1]. Various pathogenic species of enterococci are *E. avium*, *E. faecalis*, *E. raffinosus*, *E. malodoratus*, *E. pseudoavium*, *E. solitarius*, *E. gallinarum*, *E. faecium*, *E. casseliflavus*, *E. mundtii*, *E. durans*, *E. hirae* [3]. Risk factors for infections include frequent exposure to antimicrobial agents particularly the use of vancomycin and third generation cephalosporins, decreased immunity or neutropenia, renal insufficiency, use of steroids and presence of an indwelling urinary catheter [4,5]. Vancomycin resistant enterococci infection rates are highest among critically ill patients admitted in Intensive Care Units (ICU) with limited treatment options [6]. Infections by enterococci have historically been treated with semi-permeable membrane active agents (e.g., penicillin or ampicillin) in association with an aminoglycoside (streptomycin/gentamicin); however such combination treatment has failed to work due to emergence of resistance such as HLAR, beta-lactam antibiotics resistance or vancomycin resistance [7]. There are very few data regarding enterococcal infections in Manipur [8]. Hence, this study was taken up with the objective of generating data on speciation of enterococci and their antibiogram pattern.

## MATERIALS AND METHODS

The present study was a cross-sectional study conducted in the Department of Microbiology of a Tertiary Care Hospital of North

East India, Jawaharlal Nehru Institute of Medical Sciences (JNIMS), Manipur, Imphal, India from September 2018 and August 2020. Informed written consent (prescribed format) was obtained from participating individuals and in case of minors, assent was taken from the parents/legal guardians. Approval of ethical committee was obtained from the Institutional Ethical Committee JNIMS vide no. Ac/0/IEC/JNIMS/2018/(PGT).

**Inclusion criteria:** Patients of all age groups and genders with history of urinary tract infection, presence of prolonged urinary catheterisation and wound infection attending out-patient, and in-patient department of medicine, surgery, obstetrics and gynaecology, paediatrics, orthopaedics and ICU, JNIMS were considered as study population.

**Exclusion criteria:** The specimens showing contaminants, presence of duplicate isolates, patients who refuse to take part in the study and faecal samples were excluded from the study.

Sample size was calculated by:

$$n = \frac{z^2 pq}{d^2}$$

Where, n=sample size, z=1.96 at 95% confidence interval, p=prevalence from previous study=3.53% [9], q=100-p=96.47%, d=allowable error=3%. According to formula it came 145 (approx). So, the required sample size for this study was taken as 146 enterococcal isolates.

All the clinical samples except blood were processed initially by plating on Blood agar and MacConkey Agar, and incubating at 37°C for 18-24 hours. Blood samples were inoculated first in Brain Heart

Infusion (BHI) broth, incubated at 35-37°C for five days, examined daily for microbial growth (turbidity) followed by subculture on blood agar and MacConkey agar. Identification of the enterococcal isolates were performed by adopting standard protocols such as cultural characteristics, gram stain, motility testing, catalase test, bile esculin test, salt tolerance test and  $\alpha$ -pyrrolidonyl  $\beta$ -naphthylamide test (PYR). Further, speciation was carried out using the conventional scheme of Facklam and Collins [3].

The antimicrobial susceptibility testing was performed on Mueller Hinton agar plate by the Kirby Bauer disc diffusion method using the commercially available antimicrobial 6 mm discs (Himedia, Mumbai, India). The antibiotics tested were as follows- for urinary isolates and catheter tip ampicillin 10  $\mu$ g, ciprofloxacin 5  $\mu$ g, linezolid 30  $\mu$ g, nitrofurantoin 300  $\mu$ g, vancomycin 30  $\mu$ g and teicoplanin 30  $\mu$ g. For isolates from other sites like pus, wound, blood- ampicillin 10  $\mu$ g, linezolid 30  $\mu$ g, vancomycin 30  $\mu$ g and teicoplanin 30  $\mu$ g. High level gentamycin (120  $\mu$ g) and high level streptomycin (300  $\mu$ g) were used for all isolates [10].

**Quality control:** Every batch of media prepared was checked for sterility for 24 hours. Potency of disk used was checked with *Enterococcus faecalis* ATCC 29212.

## STATISTICAL ANALYSIS

Data was analysed using descriptive statistics (percentage and proportion).

## RESULTS

During the study period of two years, 146 enterococci were recovered from 14114 different clinical samples, accounting for an infection rate of 1.03% of which 71 (49%) were from inpatient, 60 (41%) from out-patient and 15 (10%) from ICU. Among 146 enterococcal isolates, maximum number of isolates were obtained from urine 116 (79.5%) and minimum number from catheter tip 3 (2.1%) [Table/Fig-1]. The predominant isolates were *E. faecalis* (82.2%) followed by *E. faecium* (15.9%) and other species were *E. durans* (1.3%) and *E. gallinarum* (0.7%). Among the ICUs highest enterococcal infection was observed in Surgical Intensive Care Unit (SICU) 46.7% followed by Medicine Intensive Care Unit (MICU) 40% and Paediatric Intensive Care Unit (PICU) 13.3% [Table/Fig-2]. The distribution of *Enterococcus* spp. in various other departments is shown in [Table/Fig-3,4].

<i>Enterococcus</i> species (N=146)	Urine (%)	Blood (%)	Pus (%)	Wound swab (%)	Catheter tip (%)
<i>E. faecalis</i> (n=120)	100 (83.3)	6 (5)	9 (7.5)	3 (2.5)	2 (1.7)
<i>E. faecium</i> (n=23)	14 (60.9)	6 (26)	1 (4.3)	1 (4.3)	1 (4.3)
<i>E. durans</i> (n=2)	1 (50)	1 (50)	0	0	0
<i>E. gallinarum</i> (n=1)	1 (100)	0	0	0	0
Total (n=146)	116 (79.5)	13 (8.9)	10 (6.8)	4 (2.7)	3 (2.1)

**[Table/Fig-1]:** Total no. of Enterococcal isolates in various clinical samples.  
\*n=No. of isolates

<i>Enterococcus</i> spp. (n=146)	MICU (%)	SICU (%)	PICU (%)
<i>E. faecalis</i> (n=120)	4 (3.3)	5 (4.2)	1 (0.8%)
<i>E. faecium</i> (n=23)	2 (8.7)	2 (8.7)	1 (4.3)
<i>E. durans</i> (n=2)	0	0	0
<i>E. gallinarum</i> (n=1)	0	0	0

**[Table/Fig-2]:** Distribution of clinical isolates in ICU (n=15).  
\*n=No. of isolates, MICU: Medicine intensive care unit; SICU: Surgical intensive care unit; PICU: Paediatric intensive care unit

In this study, more number of cases was seen among female (69.2%) than male (30.8%) thus showing female predominance. The male:female ratio was found to be 1:2.2. Mean age of males was 41.8 $\pm$ 22.28 and females were 33.59 $\pm$ 18.29 [Table/Fig-5].

*E. faecalis* was found predominant in 21-30 years age group whereas *E. faecium* was highest in the age group of 31-40 years [Table/Fig-6].

<i>Enterococcus</i> spp. (n=146)	Medicine (%)	Surgery (%)	Obstetrics and Gynaecology (%)	Paediatrics (%)
<i>E. faecalis</i> (n=120)	40 (33.3)	5 (4.2)	5 (4.2)	4 (3.33)
<i>E. faecium</i> (n=23)	3 (13.0)	0	3 (13.0)	0
<i>E. durans</i> (n=2)	0	0	0	0
<i>E. gallinarum</i> (n=1)	0	0	0	0

**[Table/Fig-3]:** Distribution of clinical isolates in OPD (n=60).  
n=No. of isolates

<i>Enterococcus</i> spp.	Medicine (%)	Surgery (%)	Obstetrics and Gynaecology (%)	Paediatrics (%)	Orthopaedics (%)
<i>E. faecalis</i> (n=120)	31 (25.8)	12 (10.0)	1 (0.8)	10 (8.3)	2 (1.7)
<i>E. faecium</i> (n=23)	5 (21.7)	3 (13.0)	1 (4.3)	3 (13.0)	0
<i>E. durans</i> (n=2)	1 (50)	0	0	1 (50)	0
<i>E. gallinarum</i> (n=1)	1 (100)	0	0	0	0

**[Table/Fig-4]:** Distribution of clinical isolates in IPD (n=71).  
n=No. of isolates

Age groups (years)	No. of samples collected (%)	Gender	
		Males (%)	Females (%)
1-10	16 (10.9)	6 (13.3)	10 (10.0)
11-20	18 (12.3)	3 (6.7)	15 (14.8)
21-30	30 (20.5)	5 (11.1)	25 (24.7)
31-40	26 (17.8)	6 (13.3)	20 (19.8)
41-50	18 (12.3)	7 (15.6)	11 (10.8)
51-60	16 (10.9)	8 (17.8)	8 (7.9)
61-70	17 (11.6)	6 (13.3)	11 (10.8)
71-80	4 (2.7)	3 (6.7)	1 (0.9)
81-90	1 (0.7)	1 (2.2)	0 (0)
Total	146	45 (100)	101 (100)
Mean age with standard deviation		41.8 $\pm$ 22.28	33.59 $\pm$ 18.29

**[Table/Fig-5]:** Distribution of Enterococci among different age group and gender.

On studying the antibiotic susceptibility pattern it was found that most of *Enterococcus* isolates were predominantly resistant to ampicillin and ciprofloxacin (73.9% in both) followed by 10.3% to vancomycin, 7.5% to teicoplanin and 3.4% to linezolid. Out of the 120 *E. faecalis* isolates, 56 (46.6%) showed HLGR and HLSR in 76 (63.3%), whereas among 23 *E. faecium* HLSR was found in 18 (78.2%) isolates and HLGR in 12 (52.1%) as shown in [Table/Fig-7].

## DISCUSSION

Enterococci are emerging as one of the most common agents of hospital acquired infection. At this context, there is a need to isolate, identify, speciate enterococci, study their antibiogram among the clinical isolates. In this study, enterococcal strains constituted 1.03% of infection. The prevalence of enterococcal infection in other Indian studies were 1.49% (Taneja N et al.), from a hospital in western India, 1.16% (Mendiratta DK et al.), from a rural hospital of Central India and 3.5% (Mathur S) from a tertiary care hospital of Northern India [9,11,12]. [Table/Fig-8] shows few studies published from North-east India showing the prevalence of 1.01% [8], 7.47% [13].

The majority of the specimens were from inpatients (49%), which were correlated with the findings of Acharya A et al., reporting 72% of specimens from hospitalised patients and 28% from outpatients [14]. In this study, a total of 10.3% of enterococcal isolates were

Enterococcal isolates	1-10 y n=16	11-20 y n=18	21-30 y n=30	31-40 y n=26	41-50 y n=18	51-60 y n=16	61-70 y n=17	71-80 y n=4	81-90 y n=1
<i>E. faecalis</i> (n=120)	11 (9.1%)	16 (13.3%)	28 (23.3%)	18 (21.6%)	16 (13.3%)	13 (10.8%)	16 (13.3%)	3 (2.5%)	0
<i>E. faecium</i> (n=23)	4 (17.4%)	1 (4.3%)	2 (8.7%)	8 (34.8%)	2 (8.7%)	2 (8.7%)	1 (4.3%)	1 (4.3%)	1 (4.3%)
<i>E. durans</i> (n=2)	1 (50%)	1 (50%)	0	0	0	0	0	0	0
<i>E. gallinarum</i> (n=1)	0	0	0	0	0	1 (100%)	0	0	0

**[Table/Fig-6]:** Distribution of Enterococcal isolates among different age groups.

\*n=No. of isolate

Enterococcus spp.	AMP		CIP		VAN		TEI		NIT		LZ		HLG		HLS	
	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R
<i>E. faecalis</i>	30	90	27	75	107	10	105	10	60	34	117	3	64	56	44	76
<i>E. faecium</i>	7	16	3	12	17	4	21	1	10	6	21	2	11	12	5	18
<i>E. durans</i>	0	2	0	1	2	0	2	0	2	0	2	0	1	1	2	0
<i>E. gallinarum</i>	1	0	1	0	0	1	1	0	0	1	1	0	0	1	0	1
Total (n=146)	38 (26.1%)	108 (73.9%)	31 (21.2%)	88 (73.9%)	126 (84.9%)	15 (10.3%)	127 (87.6%)	11 (7.5%)	72 (60.5%)	41 (34.4%)	141 (96.6%)	5 (3.4%)	76 (52%)	70 (47.9%)	51 (34.9%)	95 (65%)

**[Table/Fig-7]:** Antimicrobial susceptibility pattern of *Enterococcus* spp. done by Kirby Bauer disc diffusion method.

Note: As, the number of isolates are not equal, all the antibiotics are not used for all isolates; n=no. of isolates, AMP: Ampicillin; CIP: Ciprofloxacin; VAN: Vancomycin; TEI: Teicoplanin; NIT: Nitrofurantoin; LZ: Linezolid; HLG: High level gentamycin; HLS: High level streptomycin; \*For urine (n=116) samples and catheter tip (n=3): AMP, CIP, LZ, NIT, VAN AND TEI were used; \*For Blood (n=13), pus (n=10), wound swab (n=4): AMP, VAN, TEI, LZ were used; \*HLG and HLS were used for all isolates; \*For Intermediate isolates (n=19): Vancomycin, Teicoplanin, Nitrofurantoin

Studies	Place	Year of publication	Prevalence of enterococcal infection (%)
Phukan C et al., [13]	Assam	2016	7.47
Damrolien S et al., [8]	Manipur	2018	1.01
Present study	Manipur	2021	1.03

**[Table/Fig-8]:** Prevalence of enterococcal infection in studies from North-east India.

from ICU patients which was concordance with the findings of Tripathi A et al., (8.3%) and Paule SM et al., (13.9%) [15,16]. Patients who are admitted in ICU are at greatest risk of acquiring nosocomial infections, partly because of serious underlying disease, and also because of life saving invasive procedure, prolonged use of invasive devices and extended hospital stay. Among the ICUs, highest enterococcal infection was observed in SICU. The reason could be as post-surgical patients have longer hospital stay due to surgical site infection, soft tissue infection and have more chances of cross contamination [17].

Present study reflected *E. faecalis* as the predominant species (82.2%) followed by *E. faecium* (15.8%), *E. durans* (1.3%) and *E. gallinarum* (0.7%). Perlada ED et al., reported similar findings with 69% *E. faecalis*, 29% *E. faecium*, and 1% each of *E. avium* and *E. durans* [18]. This study demonstrated that *E. faecalis* (83.3%) as the predominant species isolated from urine samples and *E. faecium* (26%) in blood sample. However, Mohanty S et al., reported *E. faecium* (63%) as the most common isolate in blood [19].

A higher isolation rate of 69.2% was observed among the female patients than male (30.8%) which is comparable to that of Bose S et al., with females accounting for 80.4% and males 14.6% of cases [20]. Maximum number of isolates in females was seen in the age group of 21-30 years with a mean age of 33.59±18.29 years whereas in case of male, maximum isolates were found in 51-60 years with a mean age of 41.8±22.28 years. Moreover, *E. faecalis* was found predominant in 21-30 years age group whereas *E. faecium* in 31-40 years. Telkar A et al., found that, in 0-20 year age group, enterococcal isolates were highest in both the genders. However, Suchitra JB et al., found enterococci in the age group of 25 to 65 years with a mean age of 43.04±10.8 years [21,22].

On studying the antibiotic susceptibility pattern it was found that most of *E. faecalis* isolates were highly resistant to ampicillin (75%) than *E. faecium* isolates which was contradictory to the finding of Agarwal J et al., reporting significantly higher resistance *E. faecium* isolates to ampicillin than *E. faecalis*. The reason of such contrary

could be due to the fact that more number of *E. faecalis* was isolated in our study. Enterococci are intrinsically resistant to most  $\beta$ -lactam antibiotics because of low affinity Penicillin Binding Proteins (PBP), which enable them to synthesise cell wall components [23]. The present study also demonstrated that enterococcal isolates were 73.9% resistance to ciprofloxacin, 34.4% to nitrofurantoin, 10.3% to vancomycin, 7.5% to teicoplanin and 3.4% to linezolid. Bhuyan B and Das PP reported 69.9% resistance to ciprofloxacin, 17% to nitrofurantoin, 6.6% to vancomycin and 0% to linezolid [24]. However, Zhanel GG et al., observed that none of the 300 isolates of enterococci tested were resistant to nitrofurantoin [25]. That is why, at present nitrofurantoin is used increasingly to treat nosocomial urinary tract infection caused by vancomycin resistant enterococci as it can be of utmost utility in cases of multidrug resistant strains of enterococci in urine.

### Limitation(s)

In this study, minimum inhibitory concentration and genotyping of vancomycin resistance enterococci could not be performed due to lack of infrastructure.

### CONCLUSION(S)

The present study highlights the occurrence of *E. faecalis* and *E. faecium* as the predominant enterococcal species in our health care set up. Enterococcal isolates were shown higher resistance to ampicillin, ciprofloxacin, nitrofurantoin and least to linezolid followed by teicoplanin and vancomycin. It is necessary to implement infection control measures like antimicrobial stewardship especially restricting the use of antibiotics to minimum.

Further research and progress for the vancomycin resistance enterococci are required to establish a more rational approach towards improving patient outcome.

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