



## A STUDY ON PREVALENCE & ANTIBIOGRAM OF ENTEROCOCCUS SPECIES FROM VARIOUS CLINICAL SAMPLES IN A TERTIARY CARE CENTRE.

**Vijay Prakash Singh\***

Associate Professor \*Corresponding Author

**Varuna Gupta**

Assistant Professor

**ABSTRACT** *Enterococcus* species has recently evolved as an important causal agent of opportunistic nosocomial & community acquired infections exhibiting resistance with increasing frequency to many antimicrobials. The aim of this study was to determine prevalence and antibiogram of *Enterococci* in a tertiary care hospital. This study was done in the Department of Microbiology, K.D. Medical College Hospital & Research Centre, Mathura from March 2021 to February 2022. The clinically relevant samples included urine, pus, blood, wound swabs & other body fluids collected aseptically from patients admitted in the various departments and their culture and antibiotic sensitivity were performed as per standard recommendations. Minimum inhibitory concentration (MIC) of vancomycin was determined by E test for all *Enterococci* isolates which showed resistance to Vancomycin by Kirby-Bauer Disc Diffusion method. A total of 78 isolates of *Enterococcus* were obtained from 1855 clinical samples. Hence, overall prevalence of *Enterococci* from various clinical specimens was found to be 4.2%. Among 78 isolated *Enterococcus*, 5 isolates (6.4%) were Vancomycin resistant. In antibiotic susceptibility testing, *Enterococcus* showed the maximum resistance towards Ciprofloxacin & High level Gentamicin while Linezolid & Teicoplanin showed the maximum sensitivity. Various studies have shown an increase in the resistance of *Enterococci* towards many antimicrobial agents especially vancomycin. Further emergence of multidrug resistant *Enterococci* can be reduced by increasing awareness regarding drug resistance among health care workers, following of infection control measures and judicious use of proper antimicrobials according to antimicrobial susceptibility pattern.

**KEYWORDS :** *Enterococci*, Antibiogram, Vancomycin resistant *Enterococci*

### INTRODUCTION

*Enterococci* are a part of normal faecal flora in human beings. The genus *Enterococcus* are ovoid shaped, Gram positive cocci, arranged in pairs or in short chains. They are a part of normal flora of the intestinal tract, oral cavity & vagina. Due to excessive and non-specific use of broad spectrum antibiotics, *Enterococcus* species have evolved as an important causal agent of opportunistic nosocomial & community acquired infections [1-3]. Common infections caused by *Enterococci* are intra-abdominal infections, bacteremia, infective endocarditis, pelvic, biliary tract, wound, burns and urinary tract infections [4-6]. They have become increasingly important due to their increasing resistance to different antimicrobials such as  $\beta$ -lactam antibiotics, aminoglycosides and most importantly glycopeptides like vancomycin [7,8]. In recent years, Vancomycin resistant *Enterococci* (VRE) have caused outbreaks of hospital acquired infections worldwide due to widespread antibiotics abuse [9]. This emphasizes the need to determine the accurate antimicrobial resistance patterns for *Enterococci* from various clinical specimens with special reference to vancomycin susceptibility. Hence, this study was conducted at a tertiary care centre in western Uttar Pradesh (India) to find out the prevalence & antibiogram of *Enterococci* isolated in this region.

### MATERIALS & METHODS

A prospective study was conducted over a period of one year (March 2021 to February 2022) in the Department of Microbiology, K.D. Medical College Hospital & Research Centre, Mathura, Uttar Pradesh after obtaining clearance from the institutional ethical committee. A total of 1855 clinically relevant samples like urine, pus, blood, wound swabs, throat swabs & other body fluids were collected from patients admitted in the various departments. *Enterococcus* from commensal sites were excluded from the study. All the specimens received in the bacteriology laboratory were inoculated on blood agar & Mac Conkey agar plates except urine sample. The urine samples were inoculated on CLED agar. All the inoculated agar plates were incubated at 37°C for 24-48 hrs. Presumptive identification of *Enterococcus* was done on the basis of colony characteristics, Gram's staining & catalase test. Confirmation of *Enterococcus* was done by growth in 6.5% NaCl, bile esculin hydrolysis & arabinose fermentation test [10]. Antimicrobial susceptibility testing was done by Kirby-Bauer Disc Diffusion method on Mueller Hinton agar along with a control strain of ATCC *E.faecalis* 29212, as per CLSI guidelines [11]. The antibiotic sensitivity was tested for the following antimicrobial discs from Himedia laboratories: Ampicillin (10 $\mu$ g), Penicillin (10 $\mu$ g), Vancomycin (30 $\mu$ g), High level Gentamicin (120 $\mu$ g), Teicoplanin (30 $\mu$ g), Linezolid (30 $\mu$ g), Piperacillin (100 $\mu$ g) & Ciprofloxacin (5 $\mu$ g).

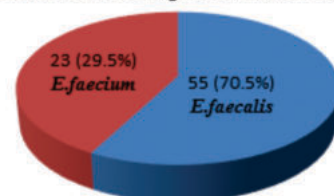
MIC for Vancomycin was done by the E test for all *Enterococci* isolates

which showed resistance to Vancomycin by Kirby-Bauer Disc Diffusion method [11].

### RESULTS

A total of 78 isolates of *Enterococcus* were obtained from 1855 clinical samples. Hence, overall prevalence of *Enterococci* from various clinical specimens was found to be 4.2% (78/1855). Out of 78, 55 (70.5%) were *Enterococcus faecalis* and 23 (29.5%) were *Enterococcus faecium* [Fig.-1]. Among 78 Enterococcal isolates, 51 were from urine samples, 22 from pus samples followed by 5 from Blood samples [Table-1]. In the study, out of 78 *Enterococci* isolates, 45 (57.7%) were from males & 33 (42.3%) from females [Fig.-2]. Among 78 isolated *Enterococcus*, 5 isolates (6.4%) were found to be Vancomycin resistant by both disk diffusion method and E test. In antibiotic susceptibility testing, *Enterococcus* showed the maximum resistance towards Ciprofloxacin & High level Gentamicin while Linezolid & Teicoplanin showed the maximum sensitivity [Table-2].

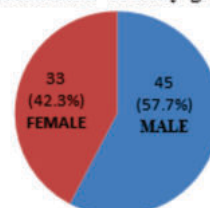
**Fig.-1: Distribution of *Enterococcus* species isolated**



**Table-1: Distribution pattern of *Enterococci* from various clinical specimens**

Specimen	Number	Percentage (%)
Urine	51	65.4
Pus	22	28.2
Blood	5	6.4

**Fig.-2: Gender wise distribution of study group**



**Table-2: Antibiotic resistance pattern of *Enterococci***

S.No.	Antibiotics	E.faecalis (n=55) Resistance	E.faecium (n=23) Resistance	Total (n=78)
1.	Ampicillin	34 (61.8%)	13 (56.5%)	47 (60.2%)
2.	Piperacillin	23 (41.8%)	10 (43.5%)	33 (42.3%)
3.	High level Gentamicin	40 (72.7%)	14 (60.8%)	54 (69.2%)
4.	Ciprofloxacin	44 (80%)	20 (86.9%)	64 (82.1%)
5.	Teicoplanin	0	0	0
6.	Vancomycin	2 (3.6%)	3 (13.04)	5 (6.4%)
7.	Linezolid	0	0	0

## DISCUSSION

*Enterococci* are part of the normal flora of gastro-intestinal tract. Although *Enterococci* were considered to be relatively harmless micro-organism in the past but in recent times, they have been isolated frequently from clinical specimens as hospital acquired pathogen [12]. The increasing resistance of *Enterococci* towards many antimicrobial agents like aminoglycosides,  $\beta$ -lactam antibiotics & glycopeptides like vancomycin have reduced the treatment options and made it an important nosocomial pathogen [12,13,14].

In the present study, *Enterococci* were isolated from various clinical specimens with prevalence rate of 4.2%. This finding is comparable with the previous study by K. Mukherjee et al [18]. Various other studies have reported varying prevalence rate [2,15].

Majority of *Enterococcus* isolates belonged to *E.faecalis* species (70.5%) followed by *E.faecium* species (29.5%) [Fig.-1]. This almost correlates with the previous studies which have reported *E.faecalis* as the major isolate of *Enterococci* [1,2,16].

Isolates were highest from urine (65.4%), followed by pus (28.2%) and blood (6.4%) [Table-1]. Other previous studies have also reported the similar findings [1,17,18].

In the present study, out of 78 *Enterococci* isolates, 57.7% were from males & 42.3% from females [Fig.-2]. This is in accordance with the study done by Jada S et al who reported *Enterococci* from 55.06% male patients & 44.94% female patients [19]. Various other studies have also reported male preponderance among patients suffering from Enterococcal infection [1,20,21].

The drug of choice for treatment of Enterococcal infection is Penicillin along with aminoglycosides. However, the increasing resistance of these antibiotics against *Enterococci* has important clinical implications. Our study showed that 60.2% of isolates were resistant to Ampicillin, 69.2% to High level Gentamicin & 82.1% to Ciprofloxacin [Table-2]. The previous studies have also reported similar findings of drastic increase in resistance of the commonly used antibiotics against *Enterococci* [1,22,23]. In our study, *Enterococcus* isolates showed 100 percent susceptibility to Linezolid & Teicoplanin which is similar to the study of Chitnis S et al [24].

Vancomycin resistance in *Enterococci* is the most recent & important resistance among all the available antimicrobial drugs. It has been increasingly reported globally. In our study, 6.4 % of the isolates are VRE. Other previous studies in the tertiary care hospitals of India have reported varying prevalence of VRE ranging from 1.7- 20 % [15]. The high prevalence of multidrug resistant Enterococcal infection in a tertiary care hospital is due to excessive & injudicious use of broad spectrum antibiotics at peripheral centres from where the patients are referred to tertiary care centres. The evolution of VRE might be attributed to imprudent use of Vancomycin & poor infection control measures.

Further emergence of VRE & multidrug resistant *Enterococci* can be reduced by increasing awareness regarding drug resistance among health care workers and judicious use of proper antimicrobials according to antimicrobial susceptibility pattern. Also, infection control measures like proper hand hygiene, use of gloves, gowns & isolation of infected patients should be strictly implemented in the health care centres.

**Conflict Of Interest:** There are no conflicts of interest.

## REFERENCES

- 1) Parameswarappa J, Basavaraj VP, Basavaraj CM. Isolation, identification and antibiogram of *Enterococcus* isolated from patients with urinary tract infection. *Ann Afr Med*. 2013;12:176-81.
- 2) Desai PJ, Pandit D, Mathur M, Gogate A. The prevalence, identification and the distribution of various species of *Enterococci* which were isolated from clinical samples, with special reference to the urinary tract infections in catheterized patients. *India J Med Microbiol*. 2001;19:132-37.
- 3) Ross PW. *Streptococci and Enterococci*. Mackie and McCartney's Practical Medical Microbiology, 14th edition. Elsevier. 2006;268-69.
- 4) Low DE, Keller N, Barth A, Jones RN. Clinical prevalence, antimicrobial susceptibility, and geographic resistance patterns of *Enterococci*: results from the SENTRY Antimicrobial Surveillance Program, 1997-1999. *Clin Infect Dis*. 2001;Suppl 2:S133-45.
- 5) Schaberg DR, Culver DH, Gaynes RP. Major trends in the microbial etiology of nosocomial infection. *Am J Med*. 1991;91(3B):72S-75S.
- 6) Koneman EW, Allen SD, Janda WM, Schreckenberger PC, Winn WC. *Koneman's Color Atlas and Textbook of Diagnostic Microbiology*. 6th Ed. Lippincott, Philadelphia. 2006.
- 7) Moellering RC Jr. Emergence of *Enterococci* as a significant pathogen. *Clin Infect Dis*. 1992;14:1173-1178.
- 8) Shah L, Mulla S, Patel KG, Rewadiwala S. Prevalence of *Enterococci* with higher resistance level in a tertiary care hospital: a matter of concern. *National J Medical Research*. 2012;2:25-27.
- 9) Pourakbari B, Aghdam KM, Mahmoudi S, Ashtiani, Sabouni F, Movahedi Z, et al. High frequency of vancomycin resistant *Enterococcus faecalis* in an Iranian referral children medical hospital. *Am J Clin Med* 2012;7:201-4.
- 10) Faeklam RR, Collins MD. Identification of *Enterococcus* species isolated from human infections by a conventional test scheme. *J Clin Microbiol*. 1989;27(4):731-4.
- 11) CLSI. Performance standards of antimicrobial susceptibility testing. 27<sup>th</sup> ed. CLSI supplement M100. Wayne PA: Clinical and Laboratory Standards Institute; 2017.
- 12) Mundy LM, Sahn DF, Gilmore M. Relationships between Enterococcal Virulence and Antimicrobial Resistance. *Clin Microbiol Rev*. 2000;13(4):513-22.
- 13) Sood S, Malhotra M, Das BK, Kapil A. Enterococcal infections & antimicrobial resistance. *Indian J Med Res*. 2008;128(2):111-21.
- 14) Murray BE. Vancomycin-resistant enterococcal infections. *N Engl J Med*. 2000;342:710-21.
- 15) Sreeja S, Sreenivasa BPR, Prathab AG. The prevalence and the characterization of the *Enterococcus* Species from various clinical samples in a tertiary care hospital. *JCDR*. 2012;6:1486-1488.
- 16) Bose S, Ghosh A K, Barapatre R. Prevalence Of Drug Resistance Among *Enterococcus* Spp Isolated From A Tertiary Care Hospital. *Int J Med Health Sci*.
- 17) Bagsaw SM, Laupland KB. Epidemiology of intensive care unit-acquired urinary tract infections. *Curr Opin Infect Dis*. 2006;19:67-71
- 18) K. Mukherjee, D. Bhattacharjee, G. Chakraborti, SS Chatterjee. Prevalence and antibiotic susceptibility pattern of *Enterococcus* species from various clinical samples in a tertiary care hospital in Kolkata. *International Journal of Contemporary Medical Research* 2016;3(6):1565-1567.
- 19) Jada S, Jayakumar K. Prevalence of *Enterococcus* species from various clinical specimens in Shri Sathyasai medical college and research institute with special reference to speciation & their resistance to vancomycin. *International Journal of Medical and Clinical Research*. 2012;3(4):154-160.
- 20) Tripathi A, Shukla SK, Singh A, Prasad KN. Prevalence, outcome and risk factor associated with vancomycin resistant *Enterococcus faecalis* and *Enterococcus faecium* at a Tertiary Care Hospital in Northern India. *Indian J Med Microbiol* 2016;34:38-45.
- 21) Manimala, E., I.M. Rejitha and Revathy, C. 2019. Detection of Vancomycin Resistant *Enterococci* in Various Clinical Sample Isolates from a Tertiary Care Centre. *Int.J.Curr.Microbiol.App.Sci*. 8(02):915-921.
- 22) Bhat KG, Paul C, Bhat MG. Neonatal bacteraemia due to high level aminoglycoside resistant (HLAR) *Enterococci*. *Indian J Pediatr*. 1997;64:537-9.
- 23) Taneja N, Rani P, Emmanuel R, Sharma M. Significance of vancomycin resistant *Enterococci* from urinary specimens at a tertiary care centre in northern India. *Indian J Med Res*. 2004;119:72-74.
- 24) Chitnis S, Katara G, Hemvani N, Pareek S, Chitnis DS. *In vitro* activity of daptomycin & linezolid against methicillin resistant *Staphylococcus aureus* & vancomycin resistant *Enterococci* isolated from hospitalized cases in Central India. *Indian J Med Res* 2013; 137: 191-6.