ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

A Study On Susceptibility Pattern Of Enterococcal Isolates From Tertiary Care Hospital: A Ray Of Hope?

Neelesh Naik[1], Uma BM[2]*, and Rama NK[3].

[1]Professor, Department of Microbiology, MVJ Medical College and Research Hospital, Bengaluru, Karnataka, India.
[2]Associate Professor, Department of Microbiology, MVJ Medical College and Research Hospital, Bengaluru, Karnataka, India.
[3]Professor, Department of Microbiology, MVJ Medical College and Research Hospital, Bengaluru, Karnataka, India.

ABSTRACT

Enterococci were organisms known to be of less virulence but the healthcare associated infections caused by them has been on the rise and the change in the antibiotic susceptibility of these organisms with rise in multidrug resistant pathogens has been a concern. This study was undertaken to know about the local antibiotic susceptibility pattern of enterococci which will be helpful in guiding therapeutic decisions. A total of 119 isolates of enterococci isolated from various clinical samples were included in the study. The identification and antibiotic susceptibility was performed using automated VITEK 2 Compact system. Among 119 enterococcus isolates, 95 (79.83%) isolates were of *Enterococcus faecalis* and 24 (20.16%) isolates were *Enterococcus faecium*. *Enterococcus* species were highly susceptible to linezolid, vancomycin and teicoplanin and were least susceptible to ciprofloxacin, levofloxacin, erythromycin, tetracycline and penicillin. Differences in susceptibility pattern between *E. faecalis* and *E. faecium* was observed for penicillin, nitrofurantoin, ciprofloxacin, levofloxacin, tetracycline and erythromycin with *E. faecium* isolates being more resistant. 7 isolates were vancomycin resistant enterococci (VRE) of which 5 were of *E. faecium* and 2 were of *E. faecalis*. VRE isolates showed maximum susceptibility to linezolid (100%) and were 100% resistant to penicillin, ciprofloxacin, levofloxacin, erythromycin and to high level gentamicin.

Keywords: Enterococci, Antibiotic susceptibility, VRE, MIC

https://doi.org/10.33887/rjpbcs/2023.14.5.19

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Enterococci are bacteria commonly residing as commensals in the intestines of humans and many other animals. They colonize the neonates early and also form a substantial part of the healthy intestinal flora of adults [1]. Enterococci were considered as low grade pathogens but the incidence of them causing healthcare associated infections has been increasing in the recent years. The genus enterococcus contains more than 20 species of which *Enterococcus faecalis* (85-90%) and *Enterococcus faecium* (5-10%) accounts for most of the human infections [2].

The most frequent infections caused by enterococci are urinary tract infections (UTI), intra abdominal and intra pelvic abscesses, surgical site infections and blood stream infections [2,3]. The increasing incidence of enterococci as healthcare associated infection causing pathogen is due to its natural ability to obtain and share extra chromosomal elements encoding virulence traits or antibiotic resistant genes [4]. Both intrinsic and acquired resistance to many antimicrobials is known to exist in *Enterococcus* spp. There are many resistance genes present that act against various antibiotics and are responsible for their intrinsic resistance to antibiotics like cephalosporins, aminoglycosides (low level resistance), clindamycin and trimethoprin- sulfamethoxazole. *E. faecalis* is the predominant species implicated in infection followed by *E. faecium* [3,5].

A standard regimen of ampicillin/ penicillin and gentamicin had been the backbone of treatment for enterococcal infections, however with the increase in resistance to these antibiotics including resistance to high level aminoglycosides the therapeutic options have become limited. Glycopeptide antibiotics like vancomycin is being used for the treatment of these infections [6]. Resistance to vancomycin was first reported in Europe in 1988 and from India in 1999 and there has been a rise in resistance since the last two decades [7]**.** Isolation of vancomycin resistant enterococci (VRE), has limited the therapeutic options and is associated with increased mortality, length of hospital stay, admission to the ICU, surgical procedures & cost [8]. It has now become a challenge to treat these infections and only a few therapeutic options, including oxazolidinones (linezolid), novel tetracyclines (tigecycline) and lipopeptides (daptomycin) remain to treat infections with multi drug resistant VRE [1]. The World Health Organization has recognized VRE as one of the most significant resistant bacteria in their "Global Priority list of antibiotic-resistant bacteria" in 2017 [7].

This study was undertaken to know about the local antibiotic susceptibility pattern of enterococci which will be helpful in incorporating the changes required in the antibiotic policy of our hospital.

MATERIALS AND METHODS

This study was done in the Department of Microbiology of a tertiary care hospital in rural Bengaluru for a period of one year between June 2022 and May 2023. A total of 119 isolates of enterococcus isolated from clinical samples from which enterococcus have got clinical significance like urine, pus, blood and body fluids were included in the study. The samples received were from patients of all age groups admitted in different clinical departments. The commensal enterococci isolated from the gastrointestinal tract, female genital tract and oral cavity, and repeat isolates from the same patient were excluded from the study.

All the specimens received in the laboratory were inoculated on blood agar and MacConkey agar plates except for urine, blood and body fluids. Urine samples were inoculated on blood agar and CLED agar and incubated at 37^0C for 24-48 hours whereas for blood and body fluids, samples were received in blood culture bottles which were immediately loaded in the automated BACT/ALERT system followed by culture of flagged positive culture bottles on 5% sheep blood agar and MacConkey agar. Presumptive identification of genus enterococcus was done on the basis of colony morphology, gram stain and catalase test. The confirmation of the organism was then performed using automated VITEK 2 Compact (BioMerieux Inc., France) according to the manufacturer's instructions.

The antibiotic susceptibility of the organisms was also performed using VITEK 2 Compact using the AST-P628 card containing benzylpenicillin, ciprofloxacin, daptomycin, erythromycin, levofloxacin, linezolid, nitrofurantoin, teicoplanin, tetracycline and vancomycin. Gentamicin high level (synergy) test

ISSN: 0975-8585

was performed using the same AST card. All the interpretations of susceptibility pattern were made according to the Clinical and Laboratory Standards Institute (CLSI) guidelines 2023 [5].

RESULTS AND DISCUSSION

The changing clinical pattern of the enterococcus infections and their antimicrobial susceptibility patterns have become an important topic of discussion, as it is emerging as a healthcare associated infection causing pathogen nowadays [9]. A total of 119 enterococci were isolated from various clinical samples in our study. Among 119 enterococcus isolates, 95 (79.83%) isolates were of *Enterococcus faecalis* and 24 (20.16%) isolates were of *Enterococcus faecium*. *E. faecalis* was the predominant species and it was similar to the observations made in other studies [3,4,10-14]. Highest prevalence of enterococcus was seen in males 76 (63.86%) followed by females 43 (36.13%) with M:F ratio of 1.76:1. Male preponderance was also observed in the studies by Sikda S and Nautival S [4,15]**.** The maximum percentage of isolation was seen among the age group of 41-60 years (32.77%). [Table 1]. In other studies maximum enterococcal isolates were from different age groups. Sharma S observed more isolates in the age group of 40-60 years similar to our study [12]. Sikda S observed more in patients above the age group of 60 years whereas Nautival S observed more in younger age group [4,15].

Table 1: Gender and Age distribution of *Enterococcus* spp.

Variables		*E. faecalis* (n= 95)	*E. faecium* (n= 24)	Total (%)
Gender	**Male**	61	15	76 (63.86)
	Female	34	09	43 (36.13)
Age	**<20 years**	27	06	33(27.73)
	21-40 years	16	04	20 (16.80)
	41-60 years	30	09	39 (32.77)
	>60 years	22	05	27 (22.68)

The isolates of enterococci were predominantly found in pus specimens followed by urine, [Table 2] similar to the findings by Chaudhury U who observed more isolates from pus [16]. Other studies have observed the maximum number from urinary isolates [4,12,14,17]. Among the various clinical departments, most of the isolates were obtained from the Medicine department (31.93%), followed by Surgery (24.36%), Paediatrics (23.52%), Orthopedics (10.08%), Obstetrics and Gynecology (5.04%) and ENT (5.04%). [Table 3] Sengupta M isolated more from Medicine department (38.81%) followed by Surgery (30.19%), Paediatrics (15.09%), Orthopaedics (14.28%) and Obstetrics and Gynaecology (1.62%) [3].

Table 2: Distribution of *Enterococcus spp.* from various clinical samples

Sample	*E. faecalis*	*E. faecium*	Total (%)
Pus (46)	40	6	38.65
Urine (36)	25	11	30.25
Blood (25)	19	6	21.00
Sterile body fluids (12)	11	1	10.08
Total (119)	95	24	(100)

Table 3: Ward wise distribution of Enterococcus isolates

Ward (No of isolates)	*E. faecalis*	*E. faecium*	Total (%)
Medicine (38)	27	11	31.93
Surgery (29)	22	07	24.36
Paediatric (28)	22	06	23.52
Orthopedics (12)	12	00	10.08
OBG (6)	06	00	5.04
ENT (6)	06	00	5.04
Total (119)	95	24	(100)

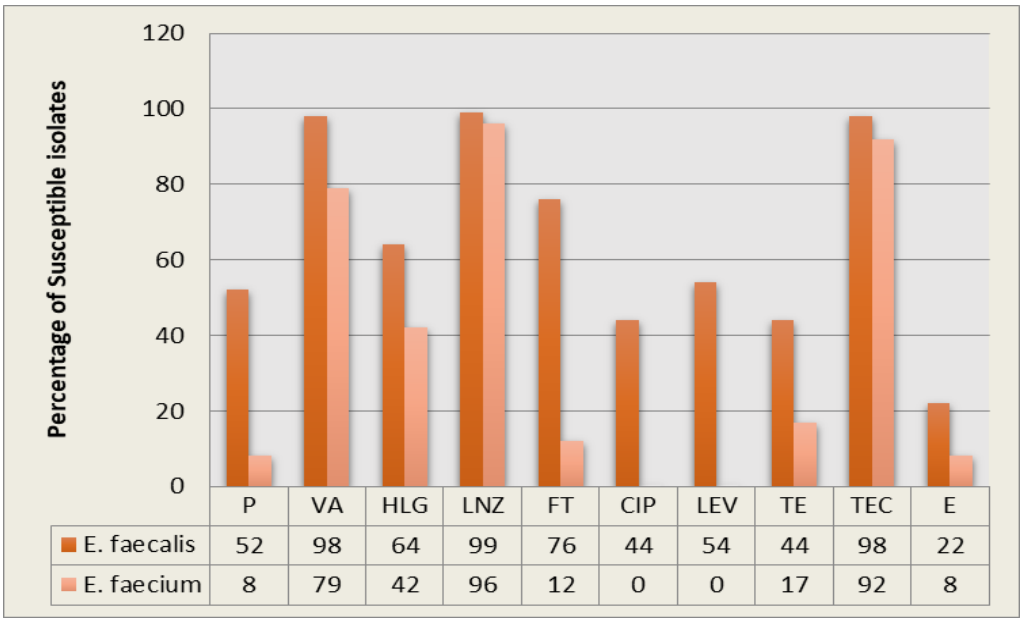
ISSN: 0975-8585

Among these 119 isolates, *Enterococcus* species were highly susceptible to linezolid, vancomycin and teicoplanin and were least susceptible to ciprofloxacin, levofloxacin, erythromycin, tetracycline and penicillin. Other studies have also found the enterococcus isolates to be more susceptible to linezolid, teicoplanin and vancomycin and decreased susceptibility to penicillin, ampicillin and ciprofloxacin [2-4,18]. 51 isolates were susceptible to penicillin and hence around 40% of enterococcal isolates in our hospital can be considered to be predictably susceptible to ampicillin, amoxicillin, ampicillin-sulbactum, amoxicillin-clavulanate and piperacillin-tazobactum [5]. The susceptibility results for daptomycin were available only for vancomycin susceptible *E. faecalis* isolates due to the absence of FDA validation to the manufacturer for providing the susceptibility results to daptomycin for *E.faecium* and VRE (vancomycin resistant enterococci) isolates. Differences in the susceptibility pattern between *E. faecalis* and *E. faecium* was observed for penicillin, nitrofurantoin, ciprofloxacin, levofloxacin, tetracycline and erythromycin with *E. faecium* isolates being more resistant. [Table 4, Graph 1] Sengupta M compared the susceptibility of *E. faecalis* and *E.faecium* and the significant difference in susceptibility was noticed towards ampicillin and nitrofurantoin only [3].

Table 4: Antibiotic susceptibility pattern of Enterococcci

Antibiotic	All Enterococci (119)			*E. faecalis* (n=95)			*E. faecium* (n=24)		
	S No (%)	I No (%)	R No (%)	S No (%)	I No (%)	R No (%)	S No (%)	I No (%)	R No (%)
Penicillin	51(43)	-	68(57)	49(52)	-	46(48)	2(8)	-	22(92)
Vancomycin	112(94.1)	-	7(5.8)	93(98)	-	2(2)	19(79)	-	5(21)
Gentamicin (high level)	71(60)	-	48(40)	61(64)	-	34(36)	10(42)	-	14(58)
Linezolid	117(98.3)	-	2(1.6)	94(99)	-	1(1)	23(96)	-	1(4)
Nitrofurantoin	75(63)	20(17)	24(20)	72(76)	11(11)	12(13)	3(12)	9(38)	12(50)
Ciprofloxacin	42(35)	4(3)	73(62)	42(44)	3(3)	50(53)	0(0)	1(4)	23(96)
Levofloxacin	51(43)	8(7)	60(50)	51(54)	7(7)	37(39)	0(0)	1(4)	23(96)
Tetracycline	46(39)	2(1.6))	71(59)	42(44)	2(2)	51(54)	4(17)	0	20(83)
Teicoplanin	115(96.6)	-	4(3.3)	93(98)	-	2(2)	22(92)	-	2(8)
Erythromycin	23(20)	17(14)	79(66)	21(22)	14(15)	60(63)	2(8)	3(12)	19(80)

Graph 1: Antibiotic susceptibility pattern of *E. faecalis and E. faecium*



P-Penicillin, VA-Vancomycin, HLG-High level gentamicin, FT-Nitrofurantoin, CIP-Ciprofloxacin, TE-Tetracycline, TEC-Teicoplanin, E-Erythromycin

The resistance to high level gentamicin was found in 40% of isolates which correlates with the resistance pattern observed by Sikdar S and Sharma S [4,12]. 7 isolates were VRE of which 5 (21%) were of *E. faecium* and 2 (2%) were of *E. faecalis*. VRE isolates showed maximum susceptibility to linezolid and were 100 % resistant to penicillin, ciprofloxacin, levofloxacin, erythromycin and to high level gentamicin. [Table 5] Others have reported the percentages of VRE isolates as 5.21%, 6.47%, 8.6% and 8.7% [3,4,10,19]. According to Dilshad Arif 12.5% of *E. faecalis* and 57.1% of *E.faecium* were VRE [2]. 5.86% of *E. faecalis* and 3.51% of *E. faecium* were VRE according to Sengupta M [3]. VRE and linezolid resistant enterococci are an emerging global threat due to the limited options available for treatment. In our study among the VRE isolates, all were susceptible to linezolid. Linezolid resistant VRE has been observed to be 1.23% by Sikdar S and 2.8% by Kotihar [4,20]. Linezolid is an oral antibiotic with good bioavailability and is considered to be among the last resort antibiotic for the management of VRE and hence it is important to identify isolates which are resistant to linezolid and to observe the slowly changing susceptibility of enterococci to linezolid. Though linezolid resistance was not observed among VRE isolates, we had 2 (1.6%) isolates which were resistant to linezolid.

Table 5: Antibiotic susceptibility of VRE isolates

Antibiotics	Susceptibility (VRE isolates=07)		
	S No (%)	I No (%)	R No (%)
Penicillin	0 (0%)	0 (0%)	7 (100%)
Gentamicin (high	0 (0%)	-	7 (100%)
Linezolid	7 (100%)	0 (0%)	0 (0%)
Nitrofurantoin	0 (0%)	1 (14.2%)	6 (85.71%)
Ciprofloxacin	0 (0%)	0 (0%)	7 (100%)
Levofloxacin	0 (0%)	0 (0%)	7 (100%)
Tetracycline	3 (42.85%)	0 (0%)	4 (57.14%)
Teicoplanin	3 (42.85%)	0 (0%)	4 (57.14%)
Erythromycin	0 (0%)	0 (0%)	7 (100%)

The MIC_{50} and MIC_{90} of the isolates for vancomycin, linezolid and teicoplanin were found to be within the susceptible MIC breakpoints and for all the other antibiotics tested it was in the resistance breakpoint category. [Table 6]. The MIC_{50} as well as MIC_{90} for linezolid was 2µg/ml. The MIC_{50} and MIC_{90} of vancomycin in our study was 1 and 2 µg/ml respectively with all the 7 resistant isolates having an MIC over 32 µg/ml. In a study conducted in Mangalore 11 out of 13 isolates of VRE had an MIC over 8 µg/ml [10]. Dilshad Arif observed 16 VRE isolates, among which 5 showed high level resistance with MIC > 256 µg/ml [2]. Bhatt P had observed 100 % of VRE isolates having MIC over 256 µg/ml [21].

Table 6: MIC values of Enterococci

Antibiotics	Susceptibility			MIC values & No of isolates												MIC 50	MIC 90
	S (No)	I (No)	R (No)	≤ 0.12	0.25	0.5	1	2	4	8	16	32	64	128	256		
Penicillin	51	0	68	15	3	2	2	16	2	11	1	16	51			32	64
Vancomycin	112	0	7			31	63	18				7				1	2
Linezolid	117	0	2			3	35	79		2						2	2
Nitrofurantoin	75	20	24								47	28	20	10	14	32	256
Ciprofloxacin	42	4	73			25	17	4	11	62						8	8
Levofloxacin	51	8	60	10	10	12	13	6	8	60						8	8
Tetracycline	46	2	71				28	18		2	71					16	16
Teicoplanin	115	0	4			92	9	8	6			4				0.5	2
Erythromycin	23	17	79		17	6	6	1	10	79						8	8

CLSI suggests use of vancomycin, high level gentamicin, daptomycin and linezolid as tier-2 antibiotics for testing and selective reporting and in our hospital, linezolid had the maximum

susceptibility followed by vancomycin and high-level gentamicin [5]. Daptomycin though not tested against *E. faecium* and VRE, it was found to be 100% susceptible for vancomycin susceptible *E. faecalis*.

CONCLUSION

Enterococci are bacteria commonly residing in the intestines of humans and have for long been considered as pathogens with low grade virulence but their role in causing healthcare associated infections has been increasing worldwide. Our study as well as studies from different parts of India have demonstrated an increase in the resistance to commonly used antibiotics as well as emergence of resistance to vancomycin and also to last resort antibiotics like linezolid. VRE in our hospital was found to be less compared to many other Indian studies and it provides us a ray of hope to further reduce the incidence of VRE and to limit its spread by taking appropriate infection control measures including contact precautions, collection of samples for culture and susceptibility tests before the start of antimicrobial treatment, selective reporting by the laboratory and the use of reserved antibiotics only if needed after the availability of the culture reports. Awareness among the clinicians regarding the emergence of VRE and the control measures is the need of the hour and also the continuous monitoring of resistance becomes a necessity which will help in formulating policies for the empirical and definitive treatment of enterococcal infections in our hospital.

REFERENCES

[1] Bender JK, Cattoir V, Hegstad K, Sadowy E, Coque TM, Westh H, et al. Update on prevalence and mechanisms of resistance to linezolid, tigecycline and daptomycin in enterococci in Europe: towards a common nomenclature. Drug Resist Updat 2018; 40:25-39.

[2] Arif D, Tripathi A, Srivastava P, Singh SP, Bisht D. Prevalence of vancomycin resistant enterococcus in various clinical specimens in tertiary care hospital. Int J Health Sci Res 2019;9(4):22-26.

[3] Sengupta M, Sarkar R, Sarkar S, Sengupta M, Ghosh S, Banerjee P. Vancomycin andlinezolid-resistant enterococcus isolates from a tertiary care center in India. Diagnostics 2023; 13:945.

[4] Sikdar S, Sadhukhan S, Majumdar AK, Bhunia S, Sarkar S, Bhattacharjee SG. Phenotypic characterisation, virulence determination and antimicrobial resistance pattern of *Enterococcus* species isolated from clinical specimen in a tertiary care hospital in Kolkata. J Clin Diagnostic Res 2021;15(7):DC06-DC09.

[5] Clinical and laboratory standards institute. Performance standards for antimicrobial susceptibility testing. 33rd ed. CLSI supplement M100. Clinical and Laboratory Standards Institute, USA, 2023.

[6] Vaghela GM, Patel RB, Shah M. A descriptive study of vancomycin resistant enterococci in tertiary care hospital of western India. Indian J Microbiol Res 2020;7(3):230-236.

[7] Sivaradjy M, Gunalan A, Priyadarshi K, Madigubba H, Rajshekar D, Sastry AS. Increasing trend of vancomycin-resistant enterococci bacteremia in a tertiary care hospital of south India: A three-year prospective study. Indian J Crit Care Med 2021;25(8):881–885.

[8] Saraswathy MP. Multidrug resistant enterococci isolated from urine samples at a tertiary care hospital. Indian J Microbiol Res 2015;2(4):214-219.

[9] 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 Microbial 2001;19:132-37.

[10] Fernandes SC, Dhanashree B. Drug resistance and virulence determinants in clinical isolates of *Enterococcus* species. Indian J Med Res 2013;137:981-85.

[11] Bose S, Ghosh A, Barapatre R. Prevalence of drug resistance among *Enterococcus* species isolated from a tertiary care hospital. Int J Med Health Sci 2012; 1:38-44.

[12] Sharma S, Gupta P, Rishi S. Prevalence of vancomycin resistant enterococcus and its antimicrobial resistance pattern in clinical isolates. Int J Med Health Res 2018;12(4):94-96.

[13] Mule P, Patil N, Gaikwad S. Medically Important *Enterococcus* species from isolates of urinary tract infection with their antimicrobial susceptibility. Int J Curr Adv Res 2018;07(5):12506-12.

[14] Bose M, Chatterjee SS, Mukherjee K, Das S, Ghosh C, Chakraborty B, et al. Enterococcal urinary taract infection: An emerging threat. J Evol Med Dent 2015;4(17):2898-904.

[15] Nautiyal S, Jauhari S, Joshi G, Kataria VK. Characterization of *Enterococcus* species in a tertiary care hospital. IOSR J Pharm 2016;6(8):48-51.

[16] Chaudhary U, Shamma M, Yadav A. Antimicrobial susceptibility patterns of common and unusual *Enterococcus* species isolated from clinical specimens. J Infect Antimicrob Agents 2007; 24:55–62.
[17] Mathew SK. A profile of vancomycin-resistant enterococcal infections and a comparison of resistance detection methods. Indian J Microbiol Res 2018;5(3):408-414.
[18] 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.
[19] Praharaj I, Sujatha S, Parija SC. Phenotypic & genotypic characterization of vancomycin resistant enterococcus isolates from clinical specimens. Indian J Med Res 2013; 138:549–556.
[20] Biswas PP, Dey S, Adhikari L, Sen A. Detection of vancomycin resistance in *Enterococcus* species isolated from clinical samples and feces of colonized patients by phenotypic and genotypic methods. Indian J Pathol Microbiol 2016; 59:188–193.
[21] Bhatt P, Patel A, Sahni AK, Praharaj AK, Grover N, Chaudhari CN, et al. Emergence of multidrug resistant enterococci at a tertiary care centre. Med J Armed Forces India 2015;71(2):139-144.