

PREVALENCE AND ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF ENTEROCOCCUS SPECIES ISOLATED IN CLINICAL SAMPLES FROM A TERTIARY CARE CENTRE

Dr. Sachin M Darji^{1*} Dr. Jigar R. Katwala²

^{1*}Associate professor, Microbiology department, SIMSR, Kalol ²Associate professor, Pharmacology department, SIMSR, Kalol

> *Correspondence author: Dr. Sachin M Darji *Email Id: sachindarji1409@gmail.com

Abstract

Objectives:

Enterococci is the part of normal commensals of human intestinal tract which is an important opportunistic pathogens. Arousal of antimicrobial resistance among the enterococci is the main threat to human healthy ecosystem. The present study was carried out to determine the prevalence of antimicrobial resistance among the *enterococci* i.e. Vancomycin-Resistant Enterococci (VRE) isolated from various clinical samples received from the patients admitted at tertiary care centre at all age group. Early detection, implementing infection control practices to minimize the transmission of infection and antimicrobial stewardship to minimize the arousal of antimicrobial resistance among the enterococci.

Methods:

A one year retrospective study was conducted at a tertiary care centre. Various specimens like pus, urine, blood, body fluid etc. were collected from the patients admitted at tertiary care centre of all age. Culture and antimicrobial sensitivity testing was carried out following standard guideline.

Results:

During the study period of one year, 950 samples were found to be bacterial culture positive out of which 110 (11.6%) isolates were identified as *Enterococcus species*. Among 110 enterococcal isolates, 80 (72.7%) isolates were *Enterococcus faecalis* and 30 (27.3%) were *Enterococcus faecium*. enterococci more frequently isolated from sample collected from male patient 75 (68.2%) than female patients 35 (31.8%). It was more frequently isolated in patients belongs to age group of 35-40 years (34.5%). Isolates were predominantly resistance to tetracycline (80.9%) followed by ciprofloxacin (70%), Benzyl-penicillin (38.2%), high-level aminoglycosides (36.4%), quinupristindalfopristin (5.5%) and vancomycin (3.6%), while linezolide and teicoplanin showed the maximum sensitivity. Our study detected the *E.faecium* more resistant strain to different antibiotics as scompared to *E. faecalis*.

Conclusion:

Our study reports the prevalence of *Enterococci* isolates as well as of VRE isolates. To reduce the arousal of VRE prevalence worldwide, appropriate use of antibiotics according to antimicrobial susceptibility testing should be encouraged. Efforts should also be made to reduce the transmission of VRE in the hospital.

Keywords: Enterococcus, Vancomycin-resistant Enterococci, Prevalence

Introduction:

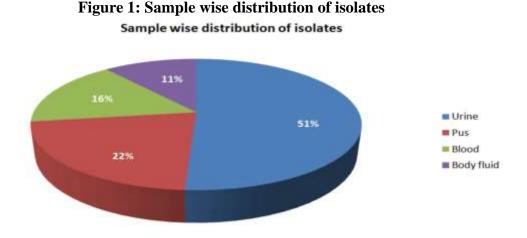
The genus *Enterococci* are Gram-positive, ovoid shaped cocci, arranged in short chain or in pairs. Though they are normal flora of the intestinal tract, oral cavity and vagina, but have emerged as nosocomial pathogens ^[1,2]. As their ability to cause different types of serious infections in human like endocarditis, bacteremia, intra-abdominal and urinary tract infection and their ability to acquired resistant to different antimicrobial agents like β lactams, aminoglycosides, glycopeptides (Vancomycin) it becomes very important to study it in details^[3]. The mainstay treatment of multiple antibiotic-resistant Gram-positive pathogen infections as Vancomycin resistant enterococci (VRE) is Linezolid, resistance to this antibiotic is now not uncommon and in fact appears to be increasing ^[4]. High mortality usually found in severe infection with *Enterococcus* which is refractory to antimicrobial therapy. Early detection of antibiotic resistance profile may help in determining a worthy alternative treatment and prevent the spread of VRE ^[5]. The main aim of our study was to identify the common species of *enterococci* causing the various infections i.e. *E.faecalis* and *E. faecuum* and their antimicrobial sensitivity pattern.

Materials & Methods:

A retrospective study was conducted in tertiary care centre, for a period of one year. All the samples (pus, urine, blood, body fluids, etc.) received from the indoor patients of all age groups admitted in tertiary care centre for culture and sensitivity report, the specimen like sputum, throat swab, stool, and vaginal swab were excluded from the study, as *Enterococcus* forms a part of the normal commensals at this region. Culture of the specimen was performed by inoculating the specimen on standard bacterial culture media plate and incubated at 37°C temperature for 18-24 hours ^[6]. Out of all culture positive samples, colonies were identified and their antimicrobial sensitivity testing was performed by an automated method i.e. VITEK 2 following Clinical and Laboratory Standard Institute (CLSI) guideline $2024^{[7]}$. Of the five known vancomycin resistance phenotypes, Van A and Van B are the most common and have been primarily described in *E. faecalis* and *E. faecium*. VanA-resistant strains show high-level resistance to vancomycin (minimum inhibitory concentrations [MICs], $\geq 64 \text{ mcg/ml}$) and teicoplanin (MICs, $\geq 16 \text{ mcg/ml}$), whereas Van B isolates show resistance to vancomycin (MICs, 4 to $\geq 1000 \text{ mcg/ml}$) but are susceptible to teicoplanin ^[8].

Results:

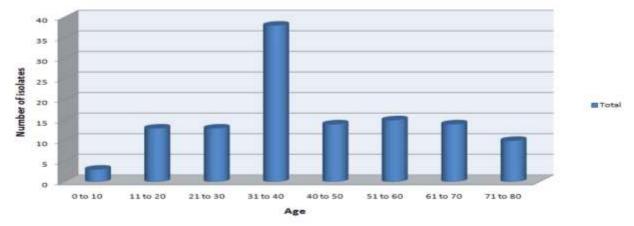
During the study period of one year, 950 samples were found to be bacterial culture positive out of which 110 (11.6%) isolates were identified as Enterococcus species. Among 110 enterococcal isolates, 80 isolates were *Enterococcus faecalis* (72.7%) and 30 were *Enterococcus faecium* (27.3%). Regarding sample wise isolation of *enterococci*, most of enterococci were isolated in the urine samples 56 (50.9%) followed by pus 24 (21.8%), blood 18 (16.4%) and body fluid 12 (10.9%) (Fig:1).



Enterococci predominantly isolated from the samples collected from male patients 75 (68.2%) than female patients 35 (31.8%). Age wise distribution showed a range from 10 months to 75 years with the maximum number of isolates 38 (34.5%) from the age group 35-40 years (Table: 01, Fig: 02).

Table 1: Age and sex wise distribution of isolates							
Age	Male	Female	Total				
0-10	2	1	3 (2.7%)				
11-20	8	5	13 (11.8%)				
21-30	9	4	13 (11.8%)				
31-40	28	10	38 (34.5%)				
41-50	10	4	14 (12.7%)				
51-60	12	3	15 (13.6%)				
61-70	10	4	14 (12.7%)				
71-80	6	4	10 (9.1%)				
Total	75	35	110				

Figure 2: Age wise distribution of isolates.



Isolates were predominantly resistance to tetracycline 89 (80.9%) followed by ciprofloxacin 77 (70%), Benzyl-penicillin 42 (38.2%), high-level-aminoglycosides (36.4%), quinupristin-dalfopristin (5.5%) and vancomycin (3.6%), while linezolide and teicoplanin showed the maximum sensitivity. Out of 4 vancomycin resistant enterococci (VRE) strain 01 (1.3%) was *E.faecalis* and 03 (10%) were *E. faecium* (Table: 02). Our study detected the *E.faecium* more resistance strain to different antibiotics as compared to *E. faecalis*.

Species	Benzylpenicillin		Ciprofloxacin		High level Gentamicin		l Tetracycline		Vancomycin		Linezolid		Teicoplanin		Quinupristin /Dalfopristin	
	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R
E.faecalis	50	30 (3.75%)	25	55 (68.8%)	60	20 (25%)	15	65 (81.3%)	79	1 (1.3%)	80	0	80	0	80	0
E.faecium	18	12 (40%)	8	22 (73.3%)	10	20 (66.7%)	6	24 (80%)	27	3 (10%)	30	0	30	0	30	6 (20%)
Total	68	42 (38.2%)	33	77 (70%)	70	40 (36.4%)	21	89 (80.9%)	106	4 (3.6%)	110	0	110	0	104	6 (5.5%)

Table 2: Antimicrobial susceptibility patterns of Enterococci

It was noted that the number of *E. faecium* isolates showing high-level aminoglycoside resistance was more as compared to *E. faecalis*, which was statistically significant (P < 0.05 by Z proportion test)

Discussion:

Enterococci is responsible for 10-20% of all hospital acquired infection^[9]. In our study, the maximum number of isolates were obtained from urine 56 (50.9%) followed by pus 24 (21.8%), blood 18 (16.4%) and body fluid 12 (10.9%)^[10,11].

Various species of *enterococci* have been identified out of which most common human infection are caused by *E.faecalis* responsible for 80-90% infections followed by *E. faecium* $5-10\%^{[12]}$. However, the recent trends show an increase in the isolation rate of *E faecium* ^[13] which is alarming as its intrinsic resistance to many antimicrobial agents may lead to a treatment failure; in our study rate of isolation of *E. faecalis* (72.7%) and *E. faecium* (27.3%).

In our study isolates were predominantly resistance to tetracycline 89 (80.9%) followed by ciprofloxacin 77 (70%), Benzylpenicilin 42 (38.2%), high-level aminoglycosides (36.4%), Quinupritin-dalfopristin (5.5%) and vancomycin (3.6%) which is in agreement with other study Chakraborty et al., 2015^[14]. There is a drastically increasing in resistant to commonly used antimicrobial agent by *enterococci* i.e.an increase in the penicillin resistance to 95%, an increase in the ampicillin resistance to 95% and an increase in the high-level aminoglycosides resistance (HLGR) to nearly 50%.^[14]

The prevalence of vancomycin resistance in the tertiary care hospitals of India has been re-ported to vary between 1.7% and 20%; in fact, in the past decade, it has been shown to be on the rise^[15,16]. Prevalence of VRE in present study is 3.6% in contrast to 13.72% in Ohri, saniya et al. 2023 ^[16]. In our study, there was 100% susceptibility to linezolid, teicoplanin, and 5.5% *Enterococcus faecium* were resistant to quinupristin/dalfopristin which is co relating with finding of study Maraki S. et al.^[17] which is in contrast to study Sengupta M et al and Sivaradjy S. et al. ^[18-24].

Conclusions:

In this study, we detected that there is a sudden increasing the cases of VRE in our healthcare setting, which is probably due to the overuse or indiscriminate usage of antibiotics and prolonged hospital stay. So early detection, treatment and preventive action will help to limit the serious consequences caused by vancomycin resistant enterococcal infection. Patients who are at high risk for colonization with VRE should be screened properly to start appropriate infection control measures that include isolation and cohorting of the VRE colonized patients, using patient-dedicated equipment, strict adherence to hand hygiene, and cleaning and disinfection of the room using appropriate chemical disinfectant after the discharge of the patient. It is also important to adhere strictly to antimicrobial stewardship protocol such as avoiding use of vancomycin for surgical antimicrobial prophylaxis, avoiding Vancomycin in empirical therapy if is not indicated, and deescalation of vancomycin empirical treatment to a susceptible narrow-spectrum antibiotic when the blood culture is negative for beta-lactam-resistant Gram-positive microorganisms.

References:

- 1. Hornuss, D., Göpel, S., Walker, S.V. : Epidemiological trends and susceptibility patterns of bloodstream infections caused by Enterococcus spp. in six German university hospitals: a prospectively evaluated multicentre cohort study. from. 20162020,
- 2. Smout, E., Palanisamy, N. & Valappil, S.P: Prevalence of vancomycin-resistant Enterococci in India between 2000 and 2022: a systematic review and meta-analysis. Antimicrob Resist Infect Control 12. 79:
- 3. Sachan, S., & Anubhaw, A. (2022: Species prevalence, antimicrobial susceptibility of enterococci isolated from various clinical samples in tertiary care hospital. International Journal of Health Sciences. 6:4507-4514.

- 4. Klare I, Fleige C, Geringer U, Thürmer A, Bender J, Mutters NT, : Increased frequency of linezolid resistance among clinical Enterococcus faecium isolates from German hospital patients. J Glob Antimicrob Resist. 2015, 3:128-31. 10.1016/j.jgar.2015.02.007. Epub 2015 Apr 13.
- 5. Yadav RK, Agarwal L: Enterococcal infections in a tertiary care hospital, North India. Ann Afr Med. 2022, 21:193-197. 10.4103/aam.aam_110_20
- 6. Collee JG, Fraser AG, Marmion BP, Simmons A: Edinburg. Churchill Livingstone, 1996. pp.:263-74.
- 7. Clinical and Lboratory Standard Institute CLSI Performance standards for Antimicrobial susceptibility testing M100. (2022). https://clsi.org/M100edition 34th 2022.
- 8. Cetinkaya Y, Falk P, Mayhall CG: Vancomycin-resistant enterococci. Clin Microbiol Rev. 2000, 13:686-707. 10.1128/CMR.13.4.686
- 9. Arias CA, Murray BE: The rise of the Enterococcus: Beyond vancomycin resistance. Nat Rev Microbiol. 2012, 10:266-78. 10.1038/nrmicro2761.
- 10. Agarwal J, Kalyan R, Singh M: High-level aminoglycoside resistance and beta-lactamase production in enterococci at a tertiary care hospital in India. Jpn J Infect Dis. 2009, 62:158-9.
- 11. Preeti S, Raman M, Nirwan PS, Meeta S, Dahiya SS: Prevalence and antimicrobial susceptibility of Enterococcus Species isolated from different clinical samples in a tertiary care hospital of North India. Natl J Med Res. 2012, 3:389-91.
- 12. Sood S, Malhotra M, Das BK, Kapil A: Enterococcal infections and antimicrobial resistance. Indian J Med Res. 2008, 128:111-21.
- 13. 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.
- 14. Chakraborty A, Pal NK, Sarkar S, Gupta MS: Antibiotic resistance pattern of Enterococci isolates from nosocomial infections in a tertiary care hospital in Eastern India. J Nat Sci Biol Med. 2015, 6:394-7.
- 15. Purohit G, Gaind R, Dawar R, Verma PK, Aggarwal KC, Sardana R,: Characterization of vancomycin resistantenterococci in hospitalized patients and role of gut colonization. J Clin Diagn Res. 201711, 1:5. 10.7860/JCDR/2017/25988.10548
- 16. Ohri, saniya & singh, kanwardeep & sidhu, shailpreet & oberoi, loveena. (2023). prevalence and antimicrobial resistance in enterococcus species. Asian Journal of Pharmaceutical and Clinical Research. 36-39. 10.22159/ajpcr.2023.v16i6.47283
- 17. Maraki S, Samonis G, Dimopoulou D, Mantadakis E: Susceptibility of Glycopeptide-Resistant Enterococci to Linezolid, Quinupristin/dalfopristin, Tigecycline and Daptomycin in a Tertiary Greek Hospital. Infect Chemother. 2014, 46:253-256.
- 18. Sengupta M, Sarkar R, Sarkar S, Sengupta M, Ghosh S, Banerjee P: Vancomycin and Linezolid-Resistant Enterococcus Isolates from a Tertiary Care Center in India. Diagnostics (Basel. 2023, 2:945-10. 10.3390/diagnostics13050945
- 19. Sivaradjy M, Gunalan A, Priyadarshi K, Madigubba H, Rajshekar D, Sastry AS: Increasing Trend of Vancomycinresistant Enterococci Bacteremia in a Tertiary Care Hospital of South India: A Three-year Prospective Study. Indian J Crit Care Med. 2021, 25:881-885.
- 20. Faron ML, Ledeboer NA, Buchan BW: Resistance mechanisms, epidemiology, and approaches to screening for vancomycinresistant enterococcus in the health care setting. J Clin Microbiol. 2016, 54:2436-2447. 10.1128/JCM.00211-16
- 21. Khandelwal N, Panwala T, Patel JS: Prevalence of enterococcus species and its vancomycin resistance pattern in a Tertiary Care Hospital, Surat, Gujarat, India: a growing threat. Int J Recent Sci Res. 2020, 11:3. 10.24327/ijrsr.2020.1107.5480
- 22. Mathis B, Haïne M, Girard R, Bonnefoy M: Risk factors for vancomycinresistant enterococcus acquisition during a large outbreak in patients aged 65 years and older. BMC Geriatr. 2019, 1186:12877-019. 10.1186/ s12877-019-1398-2

- 23. Monteserin N, Larson E: Temporal trends and risk factors for healthcare-associated vancomycin-resistant enterococci in adults. J Hosp Infect. 2016, 94:236-241. 10.1016/j.jhin.2016.07.023
- 24. Moosavian M, Ghadri H, Samli Z: Molecular detection of vanA and vanB genes among vancomycin-resistant enterococci in ICUhospitalized patients in Ahvaz in southwest of Iran. Infect Drug Resist. 2018, 11:2269-2275. 10.2147/IDR.S177886