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Vancomycin-resistant *Enterococcus* – a study on its prevalence from different clinical samples in a rural medical college hospital

Sayani Bose[1], Rituparna De[2], Paulami Palchowdhury[3], Anirban Bhaduri[4]

[1,2]Senior Resident, [4]Associate Professor, Department of Microbiology, Tamralipto Government Medical College and Hospital, Purba Midnapore, [3]Senior Resident, Department of Microbiology, Sarat Chandra Chattopadhyay Government Medical College and Hospital, Howrah, West Bengal, India

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ABSTRACT

Background: Vancomycin-resistant *Enterococcus* (VRE) is an important cause of nosocomial infection with *Enterococcus faecium* causing most of the VRE infections. Widespread use of glycopeptides in health care facilities has led to the development of VRE and enterococcal infections with high-level resistance to aminoglycosides, beta-lactamase production and glycopeptide (including vancomycin) resistance are difficult to treat and often pose a therapeutic challenge to health care facilities. **Aims and Objectives:** This study aimed to determine the antibiotic susceptibility pattern of *Enterococcus* species from various clinical specimens and to find out the occurrence rate of vancomycin-resistant enterococci. **Materials and Methods:** This study was conducted at the Department of Microbiology of Tamralipto Government Medical College and Hospital, East Midnapore, West Bengal. A total of 688 clinical samples were the total sample size taken. Isolation and identification of *Enterococcus* spp. were done by standard microbiological procedures such as culture, Gram staining, and suitable biochemical tests were conducted. Antibiotic susceptibility testing was done by the Kirby–Bauers disc diffusion method on Mueller–Hinton agar and results were interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines 2023. Teicoplanin sensitivity was performed for those isolates showing resistance to vancomycin. For strains showing resistance to vancomycin by the Kirby–Bauers disc diffusion method, vancomycin minimum inhibitory concentrations (MIC) were performed by E-test (Hi media) with MIC breakpoints between 4 and 32 as per CLSI criteria. This was compared with the control strain of American Type Culture Collection *Enterococcus faecalis* 29212 as per CLSI 2023 guidelines. **Results:** A total of 48 *Enterococcus* isolates were obtained from 688 clinical samples; 31 (8.05%) were detected from 385 urine samples, 9 (6.72%) were detected from 134 blood samples, 5 (5.15%) were detected from 97 pus/wound swab, and 3 (4.17%) were detected from 72 bronchoalveolar lavage fluid samples. Among the 48 *Enterococcus* isolates, 13 (27.08%) were vancomycin-resistant out of which nine were *E. faecalis* and four were *E. faecium*. *Enterococcus* species showed maximum resistance toward ciprofloxacin followed by ampicillin and maximum sensitivity toward teicoplanin and linezolid. **Conclusion:** Implementation of strict infection control measures, antimicrobial policies, and proper surveillance are required to identify, contain, and treat VRE infections to reduce mortality and morbidity.

Key words: Vancomycin-resistant *Enterococcus*; E-test; Minimum inhibitory concentrations; Nosocomial infection

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Address for Correspondence:
Dr. Anirban Bhaduri, Associate Professor, Department of Microbiology, Tamralipto Government Medical College and Hospital, Tamluk, Purba Midnapore, West Bengal, India. **Mobile:** +91-9073673120. **E-mail:** anir8365@gmail.com

INTRODUCTION

Vancomycin-resistant *Enterococcus* (VRE), first reported in the world in 1986 from France and first reported from India in 1999,[1] has emerged as one of the leading causes of nosocomial or health-care-associated infection and is considered a serious problem in health-care settings globally.[2] Out of all the species of Enterococci, *Enterococcus faecium* accounts for most of the VRE infections and is also the most virulent among them.[3] Enterococci can cause a variety of infections with urinary tract infections being the most common followed by bacteremia, intraabdominal infections, wound, and soft-tissue infections, neonatal sepsis, and rarely meningitis.[4]

Widespread use of glycopeptides in health-care facilities has led to the development of VRE[5] and several studies show that infection with VRE is associated with a prolonged hospital stay, increased exposure to antimicrobials particularly vancomycin, reduced immunity, renal insufficiency, steroids use and presence of indwelling urinary catheter.[6,7] Enterococcal infections with high-level resistance to aminoglycosides, beta-lactamase production, and glycopeptide (including vancomycin) resistance are difficult to treat and often pose a serious therapeutic challenge to clinicians as well as to health-care facilities.[8]

This study was conducted to find the prevalence of vancomycin resistance among *Enterococcus* isolates from various clinical specimens at a tertiary care hospital in West Bengal.

Aims and objectives

This study aimed to determine the antibiotic susceptibility pattern of *Enterococcus* species from various clinical specimens and to find out the occurrence rate of vancomycin-resistant enterococci.

MATERIALS AND METHODS

This is a hospital-based cross-sectional study conducted in the Department of Microbiology at Tamralipto Government Medical College and Hospital, East Midnapore, West Bengal, over a period of 1 year from August 2023 to July 2024.

Various clinical samples such as blood, urine, pus/wound swabs, and bronchoalveolar lavage (BAL) fluid collected aseptically from patients of all age groups admitted in various departments were received for aerobic culture in the Department of Microbiology. The sample size taken was 688 and each consecutive sample which came to our laboratory was included in our study. Urine samples were inoculated on CLED agar whereas the other samples were inoculated on blood agar and MacConkey agar and incubated at 37°C for 24–48 h. Exclusion criteria were the samples which were rejected due to faulty collection procedures and non-labeled samples.

Identification of enterococci was done by its colony characters, Gram stain, and catalase test followed by confirmation of diagnosis by Bile Esculin hydrolysis test and growth in 6.5% NaCl. *Enterococcus faecalis* and *E. faecium* were differentiated based on hippurate hydrolysis and sugar fermentation tests such as glucose, sucrose, mannitol, sorbitol, arabinose, raffinose, and pyruvate fermentation tests.

Antibiotic susceptibility testing was done by Kirby–Bauers disc diffusion method on Mueller–Hinton agar taking 0.5 McFarland standard inoculum and results were interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines 2023. The Himedia antibiotic discs used were ampicillin 10 mcg, piperacillin-tazobactam 30/6 mcg, linezolid 30 mcg, vancomycin 5 mcg, teicoplanin 30 mcg, high-level gentamycin (HLG) 120 mcg, and ciprofloxacin 5 mcg; teicoplanin sensitivity was performed for those isolates showing resistance to vancomycin.

For all those strains showing resistance to vancomycin 5 mcg by Kirby–Bauers disc diffusion method, vancomycin minimum inhibitory concentrations (MICs) were performed by E-test (Hi media) with the MIC breakpoints between 4 and 32 as per CLSI criteria. This was compared with the control strain of American Type Culture Collection *E. faecalis* 29212 as per CLSI 2023 guidelines.

The results obtained were tabulated, interpreted, and analyzed in a Microsoft Office Excel worksheet.

RESULTS

A total of48 *Enterococcus* isolates were obtained from 688 clinical samples; 31 (8.05%) were detected from 385 urine samples, 9 (6.72%) were detected from 134 blood samples, 5 (5.15%) were detected from 97 pus/wound swabs, and 3 (4.17%) were detected from 72 BAL fluid samples

Table 1: Distribution of *Enterococcus* sp. among various clinical samples

Sample type	Total no of sample (n=688)	No of enterococci	Percentage
Urine	385	31	8.05
Pus/wound swab	97	5	5.15
Blood	134	9	6.72
BAL fluid	72	3	4.17

BAL: Bronchoalveolar lavage

(Table 1). Out of 48 *Enterococcus* species, 33 (68.75%) were *E. faecalis* and 15 (31.25%) were *E. faecium* (Figure 1). Among the 48 *Enterococcus* isolates, 13 (27.08%) were vancomycin-resistant out of which nine were *E. faecium* and four were *E. faecalis*; from urine samples 8 (61.54%) VRE isolates were detected, from blood samples 3 (23.08%) VRE isolates, from pus and BAL fluid samples 1 each (1.03%) and (1.39%) were detected, respectively (Table 2). In the antimicrobial susceptibility pattern, *Enterococcus* species showed maximum resistance with ciprofloxacin followed by ampicillin; on the other hand, teicoplanin and linezolid showed maximum sensitivity among the *Enterococcus* isolates tested in our study (Table 3). Figure 2 shows the picture of e test with mic of 32 of a vre strain isolated.

DISCUSSION

Enterococci, including VRE, have emerged as an important cause of health-care-associated infection and the widespread misuse and overuse of antibiotics have led to increased resistance of enterococci against different antimicrobials such as β lactams, aminoglycosides, and particularly glycopeptides such as vancomycin.[9] In our study, the maximum number of *Enterococcus* isolates were diagnosed from urine samples (8.05%), followed by blood (6.72%), pus/wound swabs (5.15%), and BAL fluid (4.17%) samples. A study by Arif et al.,[9] also showed maximum enterococcal isolates detected from urine samples; however, few previous studies[10-12] have shown different findings compared to our study.

Among the enterococcal isolates, *E. faecalis* was the major isolate (68.75%) followed by *E. faecium* (31.25%) which is similar to many previous studies[2,9,10,12] where *E. faecalis* was the major isolate. However, in a study by Sivaradjy et al.,[13] *E. faecium* was detected more than *E. faecalis* which differs from our study.

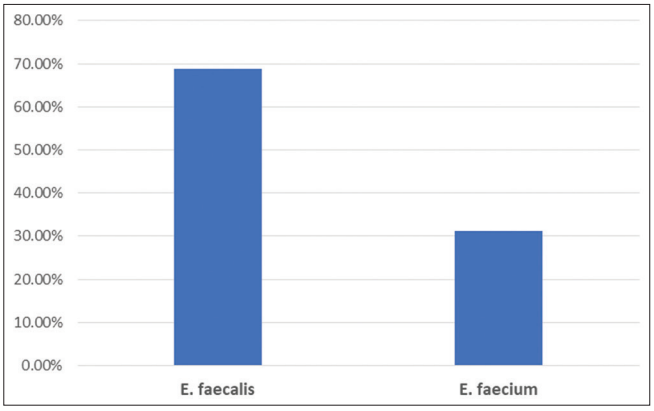

Figure 1: Distribution of isolated *Enterococcus* spp.

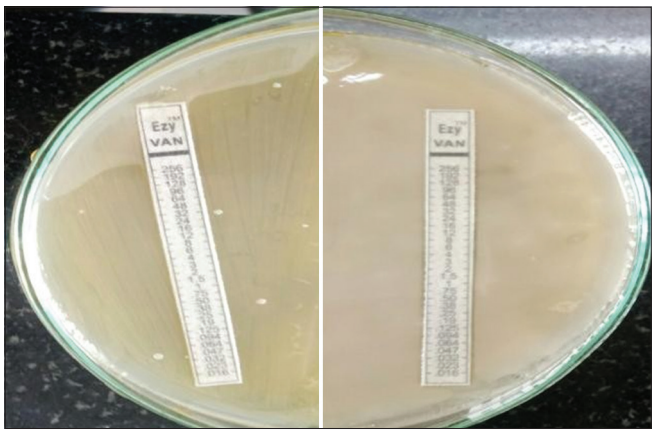

Figure 2: E-test minimum inhibitory concentrations for vancomycin-resistant *Enterococcus*

Table 2: Distribution of vancomycin-resistant *Enterococcus*

Sample	No of sample (n=688)	*Enterococcus faecium*	*Enterococcus faecalis*	No. of VRE	Percentage of VRE
Urine	385	6	3	9	2.34
Pus/wound swab	97	1	0	1	1.03
Blood	134	1	1	2	1.49
BAL fluid	72	1	0	1	1.39

BAL: Bronchoalveolar lavage

Table 3: AST pattern in VRE

Antibiotics	Resistance in *Enterococcus faecalis* (n=33) (%)	Resistance in *Enterococcus faecium* (n=15) (%)	Total (n=48) (%)
Ampicillin	22 (66.67)	12 (80)	34 (70.83%)
Piperacillin–Tazobactam	10 (30.30)	7 (46.67)	17 (35.42)
Linezolid	1 (3.03)	0 (0)	1 (2.08)
Vancomycin	4 (12.12)	9 (60)	13 (27.08)
Teicoplanin	0 (0)	0 (0)	0 (0)
High-level gentamycin	19 (57.58)	9 (60)	28 (58.33)
Ciprofloxacin	28 (84.85)	13 (86.67)	41 (85.42)

AST: Antibiotic susceptibility testing, VRE: Vancomycin-resistant *Enterococcus*

The prevalence of VRE in our study was found to be 27.08% which is a similar finding to the study by Arif et al.[9] The prevalence of VRE in our study is higher compared to a few previous studies[1,8,14,15], whereas some other studies[10,13,16,17] have reported a higher rate of VRE than our study.

Maximum number of VRE isolates was from urine (2.34%) followed by blood (1.49%), BAL fluid (1.39%), and pus/wound swab (1.03%). Other studies by Mukherjee et al.,[18] and Jada and Jayakumar[19] have shown maximum VRE isolates found in urine samples followed by pus, other body fluids, and blood samples.

In the present study, enterococcal isolates showed the highest resistance toward ciprofloxacin (85.42%) followed by ampicillin (70.83%), high-level gentamicin (58.33%), and piperacillin–tazobactam (35.42%); as discussed previously resistance toward vancomycin was 27.08%. *E. faecium* infections were found to have higher resistance toward most of the antibiotics as compared to *E. faecalis* infections; resistance toward multiple antibiotics makes the treatment of enterococcal infections more complicated. All the isolates showed maximum sensitivity toward linezolid and teicoplanin with only 2.08% resistance toward linezolid and none of the samples showed resistance toward teicoplanin.

High-level resistance toward ciprofloxacin has also been reported in studies by Mehi et al.,[20] and Narayanaswamy et al.,[21] which is a similar finding to our study. Ampicillin resistance in our study was 70.83 % which is close to the finding of a study by Mathur et al.,[22] where which showed 66% resistance to ampicillin, few other studies have also shown similar resistance against ampicillin.[18,23] HLG resistance in our study was 58.33% which is similar to that found in other studies.[9,21] Maximum sensitivity was observed toward linezolid and teicoplanin which is similar to studies by Arif et al.,[9] Mukherjee et al.,[18] and Chitnis et al.,[24] where 100% sensitivity was observed to linezolid.

Among the 13 VRE isolates found in our study, six isolates showed high-level resistance to vancomycin (MIC >256 mcg/mL) by E-test whereas other isolates showed different MIC values such as 2 mcg/mL, 4 mcg/mL, 8 mcg/mL, 16 mcg/mL, etc. which could be compared to the study by Arif et al.[9]

Limitations of study

This study has been conducted in a resource-limited setting with available diagnostic facilities. Molecular studies by PCR targeting the VRE genes would give a better result in this case.

CONCLUSION

The emergence of VRE is both a medical as well as public health problem and it is associated with multidrug-resistant infections. This highlights the importance of rapid surveillance and prompt identification, containment, and treatment of VRE infections in hospitals. Implementation of strict infection control measures, education of healthcare workers, and antimicrobial stewardship programs for rational use of vancomycin are to be strictly followed to reduce mortality and morbidity with VRE infections.

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REFERENCES

1. Smout E, Palanisamy N and Valappil SP. Prevalence of vancomycin-resistant Enterococci in India between 2000 and 2022: A systematic review and meta-analysis. Antimicrob Resist Infection Control. 2023;12(1):79.
https://doi.org/10.1186/s13756-023-01287-z
2. Tripathi A, Shukla SK, Singh A and 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(1):38-45.
https://doi.org/10.4103/0255-0857.174099
3. Viswanath LS, Sugumar M, Peela SC, Walia K and Sistla S. Detection of vancomycin Variable Enterococci (VVE) among clinical isolates of *Enterococcus faecium* collected across India-first report from the subcontinent. Indian J Med Microbiol. 2022;40(2):285-288.
https://doi.org/10.1016/j.ijmmb.2021.12.011
4. Raj HJ, Sarkar MD and Mondal S. Prevalence of enterococcal infection and the antimicrobial susceptibility profile of the organism with special reference to vancomycin: A study in a rural medical college hospital in Eastern India. IOSR J Dent Med Sci. 2019;18(1):9-15.
https://doi.org/10.9790/0853-1801010915
5. Ghoshal U, Garg A, Tiwari DP and Ayyagari A. Emerging vancomycin resistance in enterococci in India. Indian J Pathol Microbiol. 2006;49(4):620-622.
6. Boyce JM. Vancomycin-resistant *Enterococcus*. Detection, epidemiology, and control measures. Infect Dis Clin North Am. 1997;11(2):367-384
https://doi.org/10.1016/s0891-5520(05)70361-5
7. Carmeli Y, Eliopoulos GM and Samore MH. Antecedent treatment with different antibiotic agents as a risk factor for vancomycin-resistant *Enterococcus*. Emerg Infect Dis. 2002;8(8):802-807.
https://doi.org/10.3201/eid0808.010418
8. Gangurde N, Mane M and Phatale S. Prevalence of multidrug

resistant enterococci in a tertiary care hospital in India: A growing threat. Open J Med Microbiol. 2014;4(1):11-15.

https://doi.org/10.4236/ojmm.2014.41002

9. Arif D, Tripathi A, Srivastava P, Singh SP and 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.
10. Alatrouny AM, Amin MA and Shabana HS. Prevalence of vancomycin resistant enterococci among patients with nosocomial infections in intensive care unit. Al-Azhar Med J. 2020;49(4):1955-1964.

    https://doi.org/10.21608/amj.2020.120651
11. Salem-Bekhit MM, Moussa IM, Muharram MM and Hefni HM. Prevalence and antimicrobial resistance pattern of multidrug-resistant enterococci isolated from clinical specimens. Indian J Med Microbiol. 2012;30(1):44-51.

    https://doi.org/10.4103/0255-0857.93032
12. Sreeja S, Babu PR and Prathab AG. The prevalence and the characterization of the *Enterococcus* species from various clinical samples in a tertiary care hospital. J Clin Diagn Res. 2012;6:1486-1488.

    https://doi.org/10.7860/JCDR/2012/4560.2539
13. Sivaradjy M, Gunalan A, Priyadarshi K, Madigubba H, Rajshekar D and 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.

    https://doi.org/10.5005/jp-journals-10071-23916
14. Shrestha S, Kharel S, Homagain S, Aryal R and Mishra SK. Prevalence of vancomycin-resistant enterococci in Asia-a systematic review and meta-analysis. J Clin Pharm Ther. 2021;46(5):1226-1237.

    https://doi.org/10.1111/jcpt.13383
15. Vaghela GM, Patel RB and 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.

    https://doi.org/10.18231/j.ijmr.2020.042
16. Jafari-Sales A, Tarbiat-Nazloo D and ShadiDizaji A. Isolation and antibiotic resistance pattern of vancomycin-resistant *Enterococcus faecium* strains in clinical samples in Tabriz hospitals. Microbiol Curr Res. 2018;2(2):20-23.

    https://doi.org/10.4066/2591-8036.18-420
17. McDermott H, Skally M, O'Rourke J, Humphreys H and Fitzgerald-Hughes D. Vancomycin-resistant Enterococci (VRE) in the intensive care unit in a nonoutbreak setting: Identification of potential reservoirs and epidemiological associations between patient and environmental VRE. Infect Control Hosp Epidemiol. 2018;39(1):40-45.

    https://doi.org/10.1017/ice.2017.248
18. Mukherjee K, Bhattcharjee D, Chakraborti G and Chatterjee SS. Prevalence and antibiotic susceptibility pattern of *Enterococcus* species from various clinical samples in a tertiary care hospital in Kolkata. Int J Contemp Med Res. 2016;3(6):156-157.
19. Jada S and Jayakumar K. Prevalence of *Enterococcus* species from various clinical specimens in Shri Sathya Sai medical college and research institute with special reference to speciation & their resistance to vancomycin. Int J Med Clin Res. 2012;3(4):154-160.

    https://doi.org/10.9735/0976-5530.3.4.154-160
20. Mehi SK, Alam MS, Kumar R and Kumar A. Detection of vancomycin resistant enterococci in tertiary care hospital. Int J Acad Med Pharm. 2023;5(3):624-627.

    https://doi.org/10.47009/jamp.2023.5.3.130
21. Narayanaswamy A, Rajalakshmi K and Varadharajan M. Speciation and antimicrobial susceptibility pattern of enterococci from a tertiary health care center of South India. J Pharm Res. 2011;4(4):989-990.
22. Mathur P, Kapil A, Chandra R, Sharma P and Das B. Antimicrobial resistance in *Enterococcus faecalis* at a tertiary care centre of Northern India. Indian J Med Res. 2003;118:25-28.
23. Bhuyan B and Das PP. Antimicrobial resistance pattern in *Enterococcus* species and detection of vanA gene among vancomycin resistant isolates in a tertiary care hospital. Int J Med Health Res. 2018;4(2):137-140.
24. Chitnis S, Katara G, Hemvani N, Pareek S and 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(1):191-196.

Authors' Contributions:
SB- Definition of intellectual content, literature survey, prepared the first draft of the manuscript, implementation of the study protocol, data collection, and data analysis; **RD**- Design of study, statistical analysis, and interpretation; **PP-** Literature survey, preparation of tables and figures, and manuscript revision; **AB-** Concept, design, clinical protocol, manuscript preparation, editing, manuscript revision, and manuscript review.

Work attributed to:
Department of Microbiology, Tamralipto Government Medical College and Hospital, Tamluk, Purba Midnapore, West Bengal, India.

Orcid ID:
Dr. Sayani Bose - https://orcid.org/0009-0006-3578-4732
Dr. Rituparna De - https://orcid.org/0000-0002-3679-4514
Dr. Paulami Palchowdhury - https://orcid.org/0009-0009-8354-6925
Dr. Anirban Bhaduri - https://orcid.org/0000-0001-6131-4597

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