International Journal of Life Sciences, Biotechnology and Pharma Research Vol. 14, No. 4, April 2025 Online ISSN: 2250-3137
Print ISSN: 2977-0122

DOI: 10.69605/ijlbpr_14.4.2025.61

ORIGINAL RESEARCH

Prevalence of Vancomycin and Linezolid resistant Enterococci in a tertiary care hospital Karwar- A Growing concern

[1]Dr. Greeshma R Suresh, [2]Dr. Arundathi H A, [3]Dr. VN Venkatesh, [4]Dr. Sindhuja V, [5]Sneha Kukanur F, [6]Dr. Naveen G, [7]Dr. Sheetal Gouda

[1,4]2nd Year Post graduate, [2,5,7]Assistant Professor, [3]Professor and Head of the Department, [6]Associate Professor, Department of Microbiology, Karwar Institute of Medical Sciences, Karwar, Karnataka, India

Corresponding author
Dr. Sheetal Gouda
Assistant Professor, Department of Microbiology, Karwar Institute of Medical Sciences, Karwar, Karnataka, India

Received: 22 February, 2025 Accepted: 23 March, 2025 Published: 06 April, 2025

ABSTRACT

Introduction: Group D streptococci now known as enterococci have a capacity of intrinsic antibiotic resistance and also able to acquire new resistance genes and mutations. Vancomycin resistant enterococci are now becoming foremost cause for nosocomial infections. Linezolid is used as first-line of management in infections caused by vancomycin-resistant enterococcus species . Nevertheless, detection of resistance to linezolid is in increasing manner.The aim of the current study was to identify the pattern of antimicrobial resistance in Enterococcus isolates that were taken from clinical specimens at a tertiary care facility. Vancomycin and linezolid resistance were specifically targeted. **Materials and Methods:** A total of 50 samples were selected. By using standard conventional protocol like colony morphology, gram staining, catalase and bile Esculin tests. Enterococcus isolates are identified. The isolates were identified at the species level in VITEK 2 Compact (BioMeriux Inc.,France). Minimum Inhibitory Concentration of antibiotics was done in VITEK 2 COMPACT. Antimicrobial susceptibility testingwas performed by Kirby Bauer disk diffusion method. **Results:** As per our findings, the Enterococcus species isolatesobtained were Enterococcus faecalis being the most prevalent (60%), followed by Enterococcus faecium (34%) and Enterococcus avium (6%).16% of the enterococci were vancomycin-resistant.No linezolid resistance was found in our study. **Conclusion:** Isolation of enterococcus species resistant to most of the higher antibiotics like vancomycin and linezolid, from hospitalized patients at tertiary care hospital is a grave concern as such isolates have limited or no therapeutic option.Teicoplanin and other glycopeptides work better against vancomycin resistant enterococci.

Keywords: Vancomycin resistant enterococci (VRE), Linezolid, Antimicrobial susceptibility

INTRODUCTION

Gram-positive enterococci are only intestinal commensals and are not highly significant. Over the past 20 years, these have developed into lethal pathogens. Because enterococcal infections are naturally resistant to several popular antibiotics, such as β-lactams, aminoglycosides (when taken alone), cephalosporins, co-trimoxazole, and clindamycin, they are always challenging to treat.[1-2] Because of their synergistic effects, penicillin and gentamycin have been the cornerstone of enterococcal infection treatment for many years. High-level gentamycin resistance was documented by 1979 as a result of genetically acquired mechanisms, which can be either mutational or DNA acquisition. Many of the circulating strains of enterococci are now resistant to other existing therapeutic approaches due to acquired resistance.[3-4]

An oxazolidinone antibiotic called linezolid is used to treat infections brought on by gram-positive bacteria, particularly vancomycin-resistant enterococci.[3] However, it has been noted that clinical strains of enterococci are becoming resistant to linezolid. Given that the medication can be taken orally, the practice of using Linezolid indiscriminately to treat enterococcal infections hastens the emergence of Linezolid resistance.[4] Vancomycin and linezolid, which are considered to be antibiotics of last choice in enterococcal infections, are among the remaining treatment alternatives that many circulating strains are currently reported to have developed resistance too. Hospital-acquired illnesses such as urinary tract infections, surgical site infections, bacteremia,

DOI: 10.69605/ijlbpr_14.4.2025.61

endocarditis, and seldom meningitis are typically linked to enterococci.[4]

Ten thousand vancomycin-resistant enterococci-related healthcare infections occur annually in the USA alone. The second most common source of hospital-acquired infections is thought to be enterococci.[5-8]

Due to a lack of a cumulative data collection method, India's overall national enterococcal infection rate data is still unavailable. Since enterococci's ongoing evolution poses a serious threat to healthcare, a thorough grasp of their pattern of antibiotic resistance is essential. Therefore, the goal of the current study was to identify the pattern of antimicrobial resistance in Enterococcus isolates that were taken from clinical specimens at a tertiary care facility. Vancomycin and linezolid resistance were specifically targeted, and the therapeutic options for those multidrug resistant enterococcal isolates were discussed.

MATERIALS AND METHODS

It's a prospective study conducted at Department of Microbiology, Karwar institute of medical sciences, Karwar. It was conducted for a 6 months duration from January to July 2024. The study was approved by the institutional review board.All clinical samples like pus, urine, exudate, blood and body fluids received at microbiology laboratory were included. A total of 50 samples were selected. All clinical samples received at our lab were inoculated into Blood agar and MacConkey agar. The isolates were identified as Enterococcus by standard conventional protocol like colony morphology, gram staining, catalase and bile Esculin tests. The isolates were identified at the species level in VITEK 2 Compact (BioMeriux Inc.,France).

Antimicrobial susceptibility testing- Antimicrobial susceptibility testing was performed by Kirby Bauer disk diffusion method as per Clinical and Laboratory Standard Institute guidelines. Vancomycin and Linezolid resistance was tested using 30mcg disk for each as per Clinical and Laboratory Standard Institute guidelines. Minimum Inhibitory Concentration of antibiotics was done in VITEK 2 COMPACT. Vancomycin resistance resistance is considered if MIC values is more than 32 and Linezolid resistance is considered if MIC values is more than 8.

STATISTICAL ANALYSIS

Version 25.0 of the IBM SPSS® software package for Windows was used to conduct the analysis. Numbers (n) and percentages (%) were used to characterize the qualitative data.

RESULTS

A total of 50 patients' samples were selected for the study. The mean age of the patients was 32.08 years (± 28.58). In these 25 are females and 25 are males. Specimens collected for analysis include urine (36%), pus (34%), blood (26%), and bronchoalveolar lavage (4%). Basic demographic details are shown in Table 1.

Table 1: Demographic Details

Age (Mean ± SD)	32.08 ± 28.58
Sex (Male/Female)	25/25
Specimen	
Urine	18 (36.00%)
Pus	17 (34.00%)
Blood	13 (26.00%)
Bronchoalveolar Lavage	2 (4.00%)

Table 1- presents the demographic details of the study participants

As per our results the Enterococcus species isolates, obtained were Enterococcus faecalis being the most prevalent (60%), followed by Enterococcus faecium (34%) and Enterococcus avium (6%). The distribution as shown in Table 2.

Table 2: Enterococcus species isolates		
Enterococcus species	**Frequency**	**Percent**
Enterococcus faecalis	30	60.00
Enterococcus faecium	17	34.00
Enterococcus avium	3	6.00
Total	50	100.00

Table 2 displays the distribution of Enterococcus species isolates.

Among the 50 isolates, 8 were resistant to vancomycin, while 42 were sensitive. Specifically, all Enterococcus avium isolates (3) were sensitive, while 4 out of 30 Enterococcus faecalis and 4 out of 17 Enterococcus faecium isolates were resistant. The distribution as shown in Table 3.

DOI: 10.69605/ijlbpr_14.4.2025.61

Table 3: Enterococcus species vs Vancomycin resistance			
Enterococcus species	**Resistant**	**Sensitive**	**Total**
Enterococcus avium	0	3	3
Enterococcus faecalis	4	26	30
Enterococcus faecium	4	13	17
Total	8	42	50

Table 3 illustrates the vancomycin resistance patterns among different Enterococcus species.

All 50 isolates were found to be sensitive to linezolid, with no resistance observed in any of the species: Enterococcus avium, Enterococcus faecalis, or Enterococcus faecium. The distribution was shown in Table 4.

Table 4: Enterococcus species vs Linezolid resistance			
Enterococcus species	**Resistant**	**Sensitive**	**Total**
Enterococcus avium	0	3	3
Enterococcus faecalis	0	30	30
Enterococcus faecium	0	17	17
Total	0	50	50

Table 4 presents the linezolid resistance patterns among Enterococcus species.

We have compared the antimicrobial susceptibility of Enterococcus avium, Enterococcus faecalis, and Enterococcus faecium to various agents. This is shown in table 5.

Significant differences in susceptibility were observed for ampicillin, nitrofurantoin, daptomycin, and penicillin. For ampicillin, 2/3 (66.7%) of Enterococcus avium, 27/30 (90%) of Enterococcus faecalis, and 9/17 (52.9%) of Enterococcus faecium were sensitive (p=0.02). Daptomycin showed high susceptibility with 3/3 (100%) of Enterococcus avium, 30/30 (100%) of Enterococcus faecalis, and 17/17 (100%) of Enterococcus faecium (p<0.001). Nitrofurantoin exhibited significant resistance in Enterococcus avium, with 0/3 (0%) sensitivity compared to 8/30 (26.7%) in Enterococcus faecalis and 2/17 (11.8%) in Enterococcus faecium (p=0.04). Other antimicrobials like ciprofloxacin and levofloxacin showed no significant variability in resistance patterns across the species (p=0.24 and p=0.67, respectively).

Table 5: Relation of antimicrobial susceptibility among E. faecalis, E. faecium and E. avium

Antimicrobial agent (n=Sensitive)	Enterococcus avium	Enterococcus faecalis	Enterococcus faecium	p-value
Ampicillin (n=38)	2	27	9	**0.02**
Ciprofloxacin (n=6)	0	2	4	**0.24**
Levofloxacin (n=6)	0	3	3	**0.67**
High-Level Gentamycin (n=33)	3	21	9	**0.21**
Nitrofurantoin (n=10)	0	8	2	**0.04**
Daptomycin (n=50)	3	30	17	**< 0.001**
Penicillin (n=32)	2	23	7	**0.05**

Table 5 compares the antimicrobial susceptibility of Enterococcus avium, Enterococcus faecalis, and Enterococcus faecium to various agents.

DISCUSSION

As per our study, the prevalence rate of vancomycin resistant Enterococci was 16%. Out of the 50 Enterococcus isolates examined in this study, the highest percentages came from urine samples (18, 36%) and pus (17, 34%). However, in similar studies, Kanthishree BH et al. reported a relatively high rate of enterococci isolated from urine samples (72.2%)[9] and Chakraborty A et al. reported a rate of 66%.[10] Male and female isloation rates were found to be comparable in our study. However, a research by Yadav et al. revealed a high percentage of enterococci isolation from females (71%).[11] This could be because females have shorter urethras, which increases their risk of UTIs, and because the urethra is closer to the perineal area.

As earlier observed by Kanthishree BH et al., 16.6%[9] and Sharma R et al., 22%[10], pus was the second most common sample in our study (17, 34%).The most prevalent Enterococcus species in our isolates were Enterococcus faecalis (60%) and Enterococcus faecium (34%), followed by Enterococcus avium (6%). Similarly, E. faecalis was the most frequently isolated species (81.72%), according to Barman et al., from Assam.[12] E. faecium (12.9%), E. raffinosus (3.23%, n = 3), E. avium (1.08%, n = 1), and E. gallinarum (1.08%, n = 1) were next in line.[12] Of these 371 isolates, Sengupta et al.[13] found that 239 (64.42%) were Enterococcus faecalis, 114 (30.72%)

DOI: 10.69605/ijlbpr_14.4.2025.61

were Enterococcus faecium, 4 (1.08%) were Enterococcus casseliflavus, 2 (0.54%) were Enterococcus gallinarum, 4 (1.08%) were Enterococcus durans, and 8 (2.16%) were Enterococcus avium.

According to our findings, 16% of the enterococci were vancomycin-resistant. However,in contrast Phukan C et al., study showed much higher rate of VRE to be 24%; as that study only included 67 isolates, it's possible that other selection methods were used.[14]VRE rates were lower in other trials. According to Yadav et al., the VRE rate was 7%.[11] Studies by Fernandes SC et al. have revealed 8.6% VRE.[15] 8.7% for Praharaj I et al.[16] and 6.3% for Ghazawy IF et al..[17] Van A and Van B prevalences were 90.6% and 6.25%, respectively, according to Praharaj I et al..[16] . The isolation rate of VRE was 7.9%, and all of them had the Van A phenotype, in contrast to a study conducted by Tripathi A et al.[18]

The patterns of vancomycin resistance in several Enterococcus species showed that of the 50 isolates, 42 were vancomycin-sensitive and 8 were resistant. In particular, four out of thirty (13.3%) Enterococcus faecalis isolates and four out of seventeen (23.5%) Enterococcus faecium isolates were resistant, but all three (3) Enterococcus avium isolates were sensitive. All of the isolates in the Yadav et al. investigation were E. faecalis, and 14 (7%) of them had vancomycin resistance. Out of the 14 VREs that were isolated, 3 (21.4%) had Van B phenotypes and 11 (78.5%) had Van A phenotypes.[11]

The development of resistance to antibiotics like vancomycin or linezolid, which were previously thought to be a last resort, is concerning and highlights the necessity of looking into alternative treatment alternatives in these situations. Teicoplanin and other glycopeptides work better against VRE that exhibits the Van B phenotype than the Van A phenotype. However, it has been observed that isolates exhibiting the Van B phenotype develop teicoplanin resistance. Nitrofurantoin, which reaches a sufficient quantity in urine but not in blood, can be used to treat lower urinary tract infections brought on by VRE.[19]

Eight Enterococcus faecalis isolates and two Enterococcus faecium isolates in the current investigation are nitrofurantoin-sensitive. According to the current study, at least 20% of VRE-associated infections can be treated with nitrofurantoin, a less expensive and more accessible medication. This highlights the significance of culture and sensitivity in all important cases. Other substitutes, such as fosfomycin, can also be employed.Significant variations in susceptibility to ampicillin, nitrofurantoin, daptomycin, and penicillin were found in our study. 90% of Enterococcus faecalis, 52.9% of Enterococcus faecium, and 66.7% of Enterococcus avium were sensitive to ampicillin (p=0.02). 100% of Enterococcus avium, 100% of Enterococcus faecalis, and 100% of Enterococcus faecium were susceptible to daptomycin (p<0.001). With a sensitivity of 0/3 (0%) in Enterococcus avium, nitrofurantoin shown notable resistance in contrast to 8/30 (26.7%) in Enterococcus faecalis and 2/17 (11.8%) in Enterococcus faecium (p=0.04). There was no discernible variation in resistance patterns among the species for other antibiotics, such as ciprofloxacin and levofloxacin (p=0.24 and p=0.67, respectively).

The preferred medication for many types of VRE is linezolid, an oxazolidinone that was recently released in 2000 and has antibacterial efficacy by preventing the development of the 70S initiation complex. Since India reported linezolid-resistant Enterococcus in 2013, the number of linezolid-resistant VRE reports has increased.[20]

The fact that linezolid comes in both parental and oral formulations, with the oral formulation being nearly 100% bioavailable, is a significant benefit.[19] No linezolid resistance was found in our study.Yadav et al., study 2% had linezolid resistance.

The most common mechanism of linezolid resistance is mutation in the genes encoding 23S rRNA, a crucial component of the ribosome's drug binding site. This selection for mutated rRNA genes was first shown in staphylococci and has since been found in enterococci as well. It is linked to longer therapy duration. This necessitates de-escalation and appropriate use whenever linezolid is prescribed. Because it can spread between species, the other mechanism of linezolid resistance, transferable plasmid-mediated resistance to linezolid due to the cfr gene, poses a serious risk.[21] The available medications that do not have a specific VRE approval, such as chloramphenicol, doxycycline, high dose ampicillin, or ampicillin/salbactum, are available as therapy options in these situations of concurrent linezolid and vancomycin resistance.[22]

The therapy options for treating severe VRE have significantly expanded because to newer medications like daptomycin and quinupristin/dalfopristin. Daptomycin, a lipopeptide antibacterial drug, and quinupristin/dalfopristin, a mixture of streptogramin A (dalfopristin) and streptogramin B (quinupristin), work well against VRE. [19] However, quinupristin and dalfopristin have been shown to be inherently resistant to E. faecalis, making them solely effective against that species.

CONCLUSION

According to the current study, all isolates are linezolid-sensitive, and 16% of the enterococci were vancomycin-resistant. Characterizing the various resistant isolates is crucial for the appropriate handling of these cases in the current era of emerging resistance. It's also important to observe the inherent resistance of certain Enterococcus species to vancomycin.This necessitates de-escalation and appropriate use whenever linezolid is prescribed.The therapy options for treating severe VRE have

DOI: 10.69605/ijlbpr_14.4.2025.61

significantly expanded because to newer medications like daptomycin and quinupristin/dalfopristin.

Characterizing the various resistant isolates is crucial for the appropriate handling of these cases in the current era of emerging resistance. It's also important to observe the inherent resistance of certain Enterococcus species to vancomycin

REFERENCES

1. Marothi YA, Agnihotri H, Dubay D. Enterococcal resistance - An overview. Indian Journal of Medical Microbiology. 2005;23(4):214–19.
2. Washington JW, Allen S, Janda W, Konman E, Procop G, Schreckenberger P, et al. Koneman's Color Atlas and Textbook of Diagnostic Microbiology. 6th ed. Lippincott Williams and Wilkins; 2006. Streptococci, Enterococci, and the "Streptococci like" Bacteria; pp. 672–764.
3. Bhat KG, Paul C, Ananthakrishna NC. Drug resistant enterococci in a South Indian hospital. Trop Doct. 1998;28:106–07. doi: 10.1177/004947559802800220.
4. Antibiotic resistance threats in the United States, 2013. Antibiotic resistance threats in the United States, 2013 Atlanta, GA: Centres for Disease Control and Prevention, U.S. Department of Health and Human Services; 2013.
5. Mohanty S, Dhawan B, Gadepalli RS, Lodha R, Kapil A. Vancomycin-resistant Enterococcus faecium van A phenotype: first documented isolation in India. Southeast Asian Journal of Tropical Medicine and Public Health [Internet] 2006Mar [cited 2017Aug22];37(2):335–37.
6. Oberoi L, Aggarwal A. Multidrug resistant enterococci in a rural tertiary care hospital—a cause of concern. J K Science. 2010;12(3):157–58.
7. Fraser S. L. Enterococcal Infection. Medscape, Drugs, Diseases and Procedures. 2011
8. Karmarkar MG G, Edwin S, Mehta PR. Enterococcal infections with special reference to phenotypic characterization & drug resistance. Indian Journal of Medical Research. 2004;22(5):22–25.
9. Kanthishree BH, Shashikala N, Sunil DK C, Sangeetha S. Isolation, Identification And Speciation Of Enterococci And Their Antimicrobial Susceptibility In A Tertiary Care Hospital. Journal of Evolution of Medical and Dental Sciences. 2014;3(63):13893–99.
10. 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 Sc Biol Med. 2015;6:394–7. doi: 10.4103/0976-9668.160018
11. Yadav G, Thakuria B, Madan M, Agwan V, Pandey A. Linezolid and Vancomycin Resistant Enterococci: A Therapeutic Problem. J Clin Diagn Res. 2017 Aug;11(8):GC07-GC11.
12. Barman J, Nath R, Saikia L. Drug resistance in Enterococcus species in a tertiary level hospital in Assam, India. Indian Journal of Medical Research. 2016 Jan 1;143(1):107-10.
13. 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. 2023 Jan;13(5):945.
14. Phukan C, Lakhar M, Ranotkar S, Saikia KK. Emergence of vanAgene among vancomycin-resistant enterococci in a tertiary care hospital of North - East India. Indian J Med Res. 2016;143:357–61. doi: 10.4103/0971-5916.182627.
15. Fernandes SC, Dhanashree B. Drug resistance & virulence determinants in clinical isolatesof Enterococcus species. Indian J Med Res. 2013;137(5):981–85.
16. Praharaj I, Sujatha S, Parija SSC. Phenotypic & genotypic characterization of vancomycin resistant Enterococcus isolates from clinical specimens. Indian Journal of Medical Research. 2013;134(4):549–56.
17. El-Ghazawy IF, Okasha HAS, Mazloum SM. A study of high level aminoglycoside resistant enterococci. Afr J. Microbiol Res. 2016;10(16):572–77.
18. 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.
19. Kauffman CA. Therapeutic and preventative options for the management of vancomycin-resistant enterococcal infections. Journal of Antimicrobial Chemotherapy. 2003;51(Suppl 3):iii23–30. doi: 10.1093/jac/dkg273
20. Kumar S, Bandyoapdhyay M, Chatterjee M, Mukhopadhyay P, Poddar S, Banerjee P. The first linezolid-resistant Enterococcus faecium in India: High level resistance in a patient with no previous antibiotic exposure. Avicenna Journal of Medicine. 2014;4(1):13–16. doi: 10.4103/2231-0770.127416
21. Diaz L, Kiratisin P, Mendes R, Panesso D, Singh KV, Arias CA. Transferable plasmid-mediated resistance to linezolid due to cfr in a human clinical isolate of Enterococcus faecalis. Antimicrob Agents Chemother. 2012;56:3917–22.
22. Linden PK. Treatment Options for Vancomycin-Resistant Enterococcal Infections. Drugs. 2002;62(3):425–41.