e-ISSN: 0975-1556, p-ISSN:2820-2643

Available online on www.ijpcr.com

International Journal of Pharmaceutical and Clinical Research 2023; 15(8); 1549-1554

Original Research Article

Characterization and detection of Linezolid resistance in clinical isolates of Enterococci in Tertiary care Hospital of Central India

Surbhi Suneja[1], Jaya Lalwani[2], Rakesh Shrivastava[3], Deepti Chaurasia[4]

[1]Resident, Department of Microbiology, Gandhi Medical College, Bhopal
[2,3]Associate Professor, Department of Microbiology, Gandhi Medical College, Bhopal
[4]Professor and Head, Department of Microbiology, Gandhi Medical College, Bhopal

Received: 30-06-2023 / Revised: 30-07-2023 / Accepted: 26-08-2023
Corresponding author: Dr. Jaya Lalwani
Conflict of interest: Nil

Abstract:

Introduction: The prevalence of antibiotic resistance within the Enterococcus species is on the rise.

Aim: This study aimed to ascertain the occurrence of linezolid-resistance amongst the Enterococcal bacterial specimens isolated from patients.

Materials and Methods: This was a single centre, hospital; laboratory based clinical cross-sectional study conducted over a period of 18 months. The Enterococcus bacteria isolated and identified from diverse samples specimen were subjected to culture and antibiotic sensitivity testing. Identification of Enterococcus species involved conventional biochemical tests and the VITEK 2 Compact system. The antimicrobial susceptibility of the isolates to a range of antibiotics was assessed using both the Kirby–Bauer disk diffusion method and the VITEK 2 Compact system to determine their minimum inhibitory concentration (MIC). Interpretation of susceptibility was based on the guidelines from the Clinical and Laboratory Standards Institute (CLSI) 2021.

Results: A total of 645 Enterococcal isolates were included in the present study- 336 were isolated from male patients, while 309 were obtained from female patients. The majority of these isolates (24%) originated from patients aged between 19 and 30 years. Enterococci were predominantly isolated from urine samples (359 isolates - 55%), most frequently identified species was E. faecalis (52.1%). The highest prevalence of resistance was identified against Erythromycin (91%) across all species. A total of 18 (2.8%) isolates were resistant to Linezolid – of these Linezolid resistant Enterococci 15 (83.3%) were sensitive to vancomycin and 3 (17.7%) were resistant to both vancomycin and linezolid.

Conclusion: E. faecalis is stands as the prevailing clinical species extracted from clinical samples. The rise of linezolid-resistant enterococci within hospital settings sparks concern, given its role as a final treatment option for patients afflicted by vancomycin-resistant enterococci.

Keywords: Enterococcus, Linezolid resistance, Vancomycin resistant Enterococci, antibiotic susceptibility.

Introduction

Enterococci are widespread in nature and are commonly found in GI-tract of humans and other animals as well as in soil, water and food. [1] Although 35 species in genus Enterococcus have been recognized, most frequently isolated species are E.faecalis and E.faecium. [2] For many years, Enterococci were considered to be harmless resident of the gut flora, but they are now among the principal cause of health care associated infections of humans. [1] The infections of great concern are urinary tract infections, bacteraemia, endocarditis, particularly in patients with intravenous catheters, liver abscess, intra-abdominal abscess, and meningitis in neonates, central nervous system infections in adults, rarely osteomyelitis and pulmonary infections. [3,4] It is the second most common cause of UTI and ranks third amongst the hospital associated bacteria in blood stream infections in developed nations. [5,6]

The emergence of enterococci as nosocomial pathogen over two decades is mainly due to their ability to attain and transfer resistant genes, thereby developing resistance for aminoglycosides, cephalosporins, aztreonam, semisynthetic penicillin and trimethoprim-sulphamethoxazole. Enterococci possess remarkable capacity to survive in heavy antibiotic environment. Indeed, it's because of the resistance of these organisms to multiple antimicrobial agents that makes them such feared opponents. Enterococci show two types of antimicrobial resistance: intrinsic/inherent resistance and acquired resistance. Intrinsic resistance is characteristic of certain species and thus present in all members of species and is

chromosomally mediated. On the other hand, acquired resistance results from either mutation in DNA or acquisition of new DNA. [7]

Enterococci which are resistant to antimicrobial agents (aminoglycosides, penicillin, vancomycin and linezolid) pose a serious challenge not only for clinicians but also for health care institutions. MDR isolates results in treatment failure, selection and spreading of resistant strains in the health care institution. Colonization and imprudent use of antibiotics are the important causes of the multidrug resistant Enterococci. [8] Linezolid, an oxazolidinone antibiotic, was introduced early in 2000 as a new therapeutic option against gram-positive cocci, including vancomycin-resistant enterococci (VRE). Linezolid provides high level of clinical cure in complicated infections due to Enterococcus spp., including vancomycin-resistant Enterococcus fecium. Vancomycin resistant Enterococci (VRE) and Linezolid resistant Enterococci (LRE) have become a challenging nosocomial pathogen. Infections with VRE and LRE pose major therapeutic problem and leads to patient mortality and morbidity worldwide.

Though E.fecalis and E.faecium are responsible for majority of the infections, other Enterococcus species are also known to cause human infections. Recently due to their capability of causing variety of infections and difference in antimicrobial sensitivity of each species, it becomes mandatory to identify Enterococcus to species level. [9] Drug-resistant Enterococci is a challenge to the clinician and compels the clinical microbiologist to identify the appropriate antibiotic for treatment. [10] This study was primarily conducted to find out the prevalence of Linezolid resistance in enterococcal isolates in a tertiary care hospital of Central India.

Material and Methods

The study was conducted in the Department of Microbiology, Gandhi Medical College, Bhopal. Ethical clearance was taken from institutional ethical clearance committee before the commencement of the study. Identification and Speciation of Enterococci was done by conventional methods. Blood agar showing 1-2 mm small, grey, alpha, beta or non-haemolytic, translucent, circular, convex colonies with regular margins and MacConkey agar showing 0.5-1 mm tiny deep pink / magenta-coloured colonies were suspected to be Enterococci and were processed further. Suspected Enterococcal colonies were sub-cultured on Tryptic soy agar and a battery of tests was done for identification and speciation. Initially catalase test was done. Those isolates which were catalase negative were further processed for identification using Gram staining, Bile esculin hydrolysis test, Heat tolerance test and Salt tolerance test. They were further speciated using biochemical tests like, PYR test, VP test, Pyruvate utilization test, Potassium tellurite reduction test, Arginine dehydrolase test, carbohydrate fermentation tests and motility test. The various enterococcal species identified were confirmed by VITEK 2 Compact automated system.

Antibiotic susceptibility testing was performed in accordance with CLSI M-100, 31st edition (CLSI 2021) by Kirby Bauer's disk diffusion method using ampicillin (10µg), ciprofloxacin (5µg), erythromycin (15µg), high level gentamycin (120µg), doxycycline (30µg), nitrofurantoin (300µg), linezolid (30µg), teicoplanin (30µg) and vancomycin (30µg) discs. [11] Antibiotic susceptibility testing of the isolates for various antibiotics including linezolid was also performed by automated system, VITEK 2 Compact. Quality control was achieved using Staphylococcus aureus ATCC 25923, Enterococcus faecalis ATCC 29212, Escherichia coli ATCC 25922 and Pseudomonas aeruginosa ATCC 275853 for appropriate antibiotics.

Detection of Vancomycin Resistant Enterococci (VRE) was done by Kirby Bauer's disk diffusion method. The plates were incubated at 35°C ± 2°C in ambient air for a full 24 hours and was examined using transmitted light. The presence of a haze or any growth within the zone of inhibition indicates resistance. [11,12]

All the Enterococcal isolates were simultaneously screened for vancomycin resistance using vancomycin agar screen method. The BHI agar supplemented with 6µg of vancomycin/ml was prepared in-house.

Minimum inhibitory concentration (MIC) for vancomycin- resistant Enterococcus was done by Micro broth dilution (13) and also by E strip method**.** Minimum inhibitory concentration (MIC) value for Vancomycin was determined using Hi Comb MIC Strip (Hi-media, Mumbai).

Results

Out of all culture positive samples received in the Microbiology laboratory, a total of 645 Enterococcal species were isolated during the study period. Out of the total 645 Enterococcal isolates, 336 were derived from male patients, while 309 were obtained from female patients. The majority of these isolates (24%) originated from patients aged between 19 and 30 years. The age range of the patients spanned from a minimum of 2 days to a maximum of 92 years. Enterococci were predominantly isolated from urine samples (359 isolates - 55%), followed by blood samples accounting for 141 isolates (22%). Isolation from pus samples was less common, with 101 isolates (16%). Enterococcus species were detected in only 6% of sterile fluid samples, totaling 44 isolates. In this study, the most frequently identified

species was E.faecalis, accounting for 336 (52.09%) of the isolates, followed by E.faecium with 281 (43.56%) isolates. The remaining 28 (4.34%) isolates consisted of different enterococcal species, including E.avium (09 isolates), E.durans (03 isolates), E.casseliflavus (05 isolates), and E.gallinarum (11 isolates).

Table 1: Age and Sex wise distribution of the Enterococcal isolates

S. No.	Age group	Males	Females	Total
1	0 - 5 years	58	42	100
2	6 - 18 years	53	39	92
3	19 - 30 years	68	88	156
4	31 - 45 years	67	44	111
5	46 - 60 years	46	54	100
6	> 60 years	44	42	86
	Total	336	309	645

Table 2 presents the antimicrobial resistance pattern of the Enterococcal isolates. The highest prevalence of resistance was identified against Erythromycin (91%) across all species.

Ciprofloxacin exhibited a resistance rate of 85%, Ampicillin of 88%, and High-level Gentamicin of 73%. Moderate resistance was observed in Nitrofurantoin (40%) in urinary isolates, and in Doxycycline (39%). A lower level of resistance was observed for Vancomycin (11.2%), Teicoplanin (12%), and Linezolid (2.8%).

The table also highlights the dominance of resistance in E.faecium over other species, extending to all antibiotics, including Vancomycin and Teicoplanin, except for Linezolid resistance, which prevails in E.faecalis. The percentage of resistance to Linezolid is nearly equal in both E.faecalis and E.faecium. No instances of glycopeptide or Linezolid resistance were observed in other enterococcal species. Out of the 645 isolates, a total of 18 were identified as resistant to linezolid. Among these, 10 were urinary isolates, 4 originated from blood, 2 were derived from fluid samples, and 2 were pus isolates. Consequently, resistance to linezolid was notably higher in urinary isolates in comparison to isolates from blood, fluid, and pus samples.

Table 2: Resistance of Enterococcus species to different antibiotics

Organism	Ampicillin Resistant	Ciprofloxacin Resistant	Doxycycline Resistant	Erythromycin Resistant	HLG Resistant	Linezolid Resistant	Vancomycin Resistant	Teicoplanin Resistant
E.fecalis (n=336)	273 (81%)	276 (82%)	125 (37%)	306 (91%)	236 (70%)	12 (3%)	14 (4.16%)	24 (7%)
E.fecium (n=281)	242 (86%)	253 (90%)	113 (40%)	262 (93%)	214 (76%)	6 (2%)	49 (17.4%)	54 (19%)
Other Enterococci (n=28)	14 (50%)	21 (75%)	14 (50%)	24 (83%)	26 (92%)	0	9 (32.1%)	2(7%)
Total resistance (n=645)	529 (82%)	550 (85%)	252 (39%)	592 (91%)	476 (73%)	18 (2.8%)	72 (11.2%)	80 (12%)

Table 3: Cross resistance pattern between vancomycin and linezolid (n=645)

	Vancomycin Sensitive Enterococci	Vancomycin Resistant Enterococci (VRE)
Linezolid Sensitive Enterococci	558 (86.5%)	69 (10.7%)
Linezolid Resistant Enterococci	15 (2.3%)	3 (0.47%)

Table 3 presents the cross-resistance pattern between vancomycin and linezolid among a population of 645 Enterococci isolates. The majority of the total isolates (86.5%) were sensitive to both vancomycin and linezolid, indicating a susceptibility to both antibiotics. A small proportion of vancomycin sensitive Enterococci (2.61%) exhibited resistance to linezolid, which suggests a limited overlap between vancomycin sensitivity and linezolid resistance. Although, more than 95% of VRE isolates still remained sensitive to linezolid, indicating that while they were resistant to vancomycin, they retained sensitivity to linezolid. A small percentage (4.16%) of VRE isolates displayed resistance to both vancomycin and linezolid, indicating a combination of resistance to two key antibiotics. A noteworthy percentage of vancomycin-resistant isolates retained sensitivity to linezolid, implying potential treatment options for this subset. However, the occurrence of isolates resistant to both antibiotics is a cause of grave concern. These findings underscore the complexity of cross-resistance patterns between vancomycin and linezolid within the Enterococci population.

The linezolid-vancomycin resistant enterococci (LVRE) were isolated 1 each from urine, blood and cerebrospinal fluid. Isolates from blood and CSF were identified as E.faecium while isolate from urine was identified as E.faecalis using VITEK 2 compact ID and AST panel. All three LVRE isolates were vanA phenotype and van A genotype for vancomycin resistance.

Discussion

Enterococcus species have been recognized as pathogens responsible for diseases such as bacteraemia, endocarditis, complicated urinary tract infections, intra-abdominal infections, pelvic infections, wound and soft tissue infections, among others. Vancomycin-resistant enterococci (VRE) have gained significance as a cause of healthcare-associated infections due to their rapid dissemination, association with high mortality rates, and limited treatment alternatives. Over a span of one and a half years, a total of 645 Enterococci were identified from culture-positive samples. The majority of isolates were sourced from urine (55%), followed by blood (22%), pus (16%), and fluids (6%). Similar patterns were found in studies conducted by Ira Praha raj et al [14], Mathur P et al [15], Yasliani S et al [16], Sharma R et al [17], Golia S et al [18], Srivastava P et al [19], and Subendhu Sidkar et al [10] [20]. These consistent findings emphasize urinary tract infections as the primary presentation caused by Enterococci. However, Mohanty S et al (21) reported different results in 2005, where blood (36.1%) was the predominant source, followed by urine (35.2%).

Among the total Enterococcal isolates, 52.09% were identified as E.faecalis, followed by E.faecium at 43.56%. There were also 28 (4.34%) isolates of other enterococcal species, including E.avium (09), E.durans (03), E.casseliflavus (05), and E.gallinarum (11). Similar species distributions were observed in studies conducted by Ashfaq A Shah et al (2022) [22], Trupti B. Naik et al (2016) (23), Saraswathy MP (2015) [24], Mulla S et al (2012) [25], and Jain S et al (2011) [11]. Enterococci inherently resist penicillins, cephalosporins, aminoglycosides, and lincosamides. Recent instances of high-level resistance to multiple antibiotics, including aminoglycosides, ampicillin, and vancomycin, have emerged.

They are also prone to developing resistance to tetracyclines, macrolides, and chloramphenicol. Some strains may even produce beta-lactamase. Multidrug-resistant Enterococci colonization and infection have become global issues. The increased prevalence of E.faecium, particularly during the COVID-19 pandemic, raises concerns for infection control policymakers due to its higher resistance to common antibiotics, including vancomycin. In our study, resistance to all tested antibiotics was higher in E.faecium than in E.faecalis, except for Linezolid, which was slightly higher in E.faecalis (3%) compared to E.faecium (2%). Nonetheless, this observation necessitates confirmation through a larger study with more linezolid-resistant isolates.

While Enterococci are not inherently highly virulent, resistance to multiple antimicrobial drugs complicates treatment. Acquired resistance to ampicillin, aminoglycosides, and glycopeptides, notably vancomycin, exacerbates the issue. Our study shows significant resistance against Erythromycin (91%) and Ciprofloxacin (85%), aligning with findings by Srujana Mohanty et al (27]. Notably, resistance to High-level Gentamicin was found to be 73% in our study, similar to Srujana Mohanty et al [27]. A comprehensive strategy involving restricted antibiotic prescriptions and effective infection control practices seems feasible to mitigate the spread of these bacteria. Moderate resistance was observed for Nitrofurantoin (40.4%) and Doxycycline (39%). Linezolid resistance findings mirrored those of Srujana Mohanty et al

[21] (2.8% vs. 4.5%). Glycopeptide antibiotic resistance varies geographically. Our study highlights higher resistance percentages to Teicoplanin (12%) and Vancomycin (11.2%). Remarkably, 71% of VRE isolates were Teicoplanin-resistant. E.faecalis historically dominated infections, but E.faecium has been on the rise, linked to the emergence of VRE. Linezolid is an essential treatment option, though instances of resistance are concerning. Detecting isolates resistant to both vancomycin and linezolid is worrisome. The increasing prevalence of Linezolid-resistant Enterococci raises concerns, as this species exhibits higher resistance to vancomycin and other common antibiotics. Further studies are needed to validate these findings and inform effective treatment strategies. LVRE presents a challenge to clinicians as it limits available treatment options. Timely and accurate detection, as well as surveillance mechanisms, are crucial. Whole-genome sequencing may aid in identifying and addressing these emerging resistant strains.

Conclusion: Understanding the resistance pattern of the organism is crucial in developing effective empirical therapy strategies. This pattern can significantly vary across different geographical regions and healthcare settings. Within hospital environments, the prevalence of resistant organisms is notable due to the indiscriminate utilization of broad-spectrum antibiotics. This unwarranted usage perpetuates a reservoir of bacteria with resistance traits. The resistance exhibited towards commonly employed antibiotics holds greater importance than resistance towards more potent agents, as acquiring resistance to all available antibiotics could potentially regress us to a pre-antibiotic era. Given the lack of standardized antimicrobial therapies for patients infected with multidrug-resistant Enterococci, preventing the dissemination of these organisms takes on paramount significance. The emergence of Linezolid resistance is a cause for concern. Such a scenario necessitates a reduction in the inadvertent use of Linezolid and the implementation of appropriate infection control measures. These measures are essential to curtail the propagation of Linezolid-resistant pathogens, thereby safeguarding treatment options from complete loss.

References

1. Top J, Willems R, Bonten M. Emergence of CC17 Enterococcus faecium: from commensal to hospital-adapted pathogen. FEMS Immunol Med Microbiol. 2008; 52(3):297-308.
2. Rice LB. Emergence of Vancomycin resistant Enterococci Emerg Infect Dis. 2001; 7: 183-7.
3. Desai PJ, Pandit D, Mathur M, Gogate A. Prevalance, identification and distribution of various species of Enterococci isolated clinical specimens with special reference to urinary tract infections in catheterized patients. Indian J Med Microbiol. 2001; 19(3):132-7.
4. Parameswarappa J, Basavaraj VP, Basavaraj CM. Isolation, identification, and antibiogram of Enterococci isolated from patients with urinary tract infection Ann Afr med. 2013;12(3):176 81.
5. Shinde RS, Koppikar GV, Seema Oommen S. Characterization and Antimicrobial susceptibility pattern of clinical isolates of 142 Enterococci at a tertiary care hospital in Mumbai, India. Annals of Tropical Medicine and Public health. 2012; 5(2):85-8.
6. Moses V, Jerobin J, Nair A, Sathyendara S, Balaji V, George IA et al. Enterococcal bacteraemia is Associated with Prolonged Stay in the Medical Intensive Care Unit. J Glob Infect Dis. 2012; 4(1):26-30.
7. Marothi YA, Agnihotri H, Dubey D. Enterococcal resistance- An overview. Indian J Med Microbiol. 2005; 23(4):214-9.
8. Rahangdale VA, Agrawal G, Jalgaonkar SV. Study of antimicrobial resistance in enterococci. Indian J Med Microbiol. 2008; 26(3):285-7.
9. Moellering RC Jr. Emergence of enterococci as a significant pathogen. Clin Infect Dis. 1992; 14:1173-8.
10. Adhikari L. High-level aminoglycoside resistance and reduced susceptibility to vancomycin in Nosocomial Enterococci. J Glob Infect Dis. 2010; 2(3):231-5.
11. CLSI. Performance Standards for Antimicrobial Susceptibility Testing. 31st ed. CLSI supplements M100. Clinical and Laboratory Standards Institute; 2021
12. Jain S, Kumar A, Kashyap B, Kaur IR. Clinico-epidemiological profile and high-level aminoglycoside resistance in Enterococcal septicaemia from a tertiary care hospital in east Delhi. Int J Appl Basic Med Res. 2011; 1(2):80-3.
13. Programme officer. SOP-Bacteriology-BMD vancomycin susceptibility test for aerobic gram-positive cocci; NCDC National Programme on AMR containment; 31.08.2020; 1-16; 01.
14. Praharaj I, Sujatha S, Parija SC. Phenotypic & genotypic characterization of vancomycin resistant Enterococcus isolates from clinical specimens. Indian J Med Res. 2013; 138(4):549-556.
15. Mathur P, Kapil A, Chandra R, Sharma P, Das B. Antimicrobial resistance in Enterococcus faecalis at a tertiary care centre of northern India. Indian J Med Res. 2003; 118:25-8.
16. Yasliani S et al, Linezolid vancomycin resistant Enterococcus isolated from clinical samples in Tehran hospitals, 2009;7(63) 297-02.

17. Sharma R, Pai C, Prevalence of Various Enterococcal Infections and Its Antibiotic Susceptibility with Special Reference to Vancomycin and High-Level Gentamicin Resistance in a Tertiary Care Centre in Navi Mumbai, Int. J. Pharm. Sci. Rev. Res. 2013; 23(2):122-5
18. Golia S, Nirmala AR, Asha S, Kamath B, Isolation and speciation of enterococci from various clinical samples and Their antimicrobial susceptibility pattern with special reference to high Level aminoglycoside resistance, Int J Med Res Health Sci. 2014;3(3):526-9
19. Srivastava P, Mehta R, Nirwan PS, Sharma M, Dahiya SS, prevalence and antimicrobial susceptibility of Enterococcus species isolated from different Clinical samples in a tertiary care hospital of north India. 2013; 4(3)
20. 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. Journal of Clinical & Diagnostic Research. 2021 Jul 1; 15(7).
21. Mohanty S, Jose S, Singhal R, Sood S, Dhawan B, Das BK, Kapil A. Species prevalence and antimicrobial susceptibility of enterococci isolated in a tertiary care hospital of North India. Southeast Asian journal of tropical medicine and public health. 2005 Jul 1; 36(4):962.
22. Ashfaq A Shah, Syed Khursheed, Aadil Rashid, Amit Gupta, 2022. Isolation, Identification, Speciation, and Antibiogram of Enterococcus species by conventional methods and Assessment of the Prevalence of vanA genotype among VRE. Journal of Medical. Pharmaceutical and Allied Science. V 11 - I 4, Pages – 5037 - 5044. Doi: 10.55522/ jmpas. V11I4.2367.
23. Naik TB, Lavanya J, Mane V, Upadhya AK. "Enterococci speciation and antibiogram: an assessment of data at a tertiary care hospital in Karnataka. Indian J Microbiol Res 2016; 3(2):130 135.
24. Saraswathy MP, Multidrug resistant Enterococci isolated from urine samples at a tertiary care hospital. Indian J Microbiol Res 2015; 2(4):214-219.
25. Mulla S, Patel KG, Panwala T, Rewadiwala S, Prevalence of enterococci with higher resistance level in a tertiary care hospital: a matter of concern, National Journal of Medical Research. 2012 ;1(2)
26. Mundy LM, Sahm DF, Gilmore MS. Relationship between Enterococcal virulence and antimicrobial resistance. Clin Microbiol Rev. 2000, Oct; 13(4):513-22.
27. Srujana Mohanty, Bijayini Beher; Antibiogram Pattern and Virulence Trait Characterization of Enterococcus Species Clinical Isolates in Eastern India: A Recent Analysis; J Lab Physicians 2022;14:237–246
28. Patel KP, Mulla S. Speciation of Enterococcus species: better way to deal with clinical infections at resource limited settings. Int J Res Med Sci 2020; 8:941-5.