e-ISSN: 0976-822X, p-ISSN:2861-6042

Available online on http://www.ijcpr.com/

International Journal of Current Pharmaceutical Review and Research 2023; 15(2); 15-21

Original Research Article

Determination of Vancomycin, Linezolid and Daptomycin Resistance among *Eenterococcus* Isolates from a Tertiary Care Hospital

Simmi Kumari[1], Manish Kumar[2], Shilpi Singh[3]

[1]Senior Resident, Department of Microbiology, AIIMS, Patna, Bihar, India
[2]Assistant Professor, Department of Microbiology, Narayan Medical College and Hospital, Sasaram,Rohtas, Bihar, India
[3]Department of Amity School of Engineering and Technology,Amity University Patna, Bihar, India

Received: 10-12-2022 / Revised: 10-01-2023 / Accepted: 10-02-2023
Corresponding author: Dr. Manish Kumar
Conflict of interest: Nil

Abstract

Aim: The aim of the present study was to measure the prevalence of vancomycin, linezolid and daptomycin resistance in *Enterococcus* clinical isolates from patients in a tertiary hospital.

Methods: This study was a retrospective study conducted by Department of Microbiology, AIIMS, Patna, Bihar, India. Total of 500 consecutive, non-repetitive clinical isolates of *Enterococcus* species from different clinical samples were included in the study. The study period was two years.

Results: A total of 500 *Enterococcus* were isolated during the study period. Of these, there were 350 (70%) isolates were of *Enterococcus faecalis* and 150 (30%) of *Enterococcus faecium.* No other *Enterococcus* species were isolated. Clinical samples included urine, blood, pus, sputum and other samples like body fluids etc. Out of these 500 isolates, the most common sample was urine (440 (88%)), followed by blood (36 (7.20%)), pus (12 (2.4%)), and sputum (8 (1.6%)). Among isolates of *Enterococcus*- 22, 43 and 435 were intermediate, resistant and sensitive to vancomycin respectively. Linezolid intermediate and resistant was identified in 2 and 4 isolates of *Enterococcus* faecium only. MIC to vancomycin ranged between 0.25-256 µg/ml, MIC of linezolid ranged between 0.25- 16µg/ml and MIC for daptomycin was less than 1 µg/ml for all the isolates.

Conclusion: Our study, demonstrated relatively low prevalence of vancomycin and linezolid resistance, but emergence of combined newer drug resistance in *Enterococcus* species is cause of concern and reiterates the importance of importance of judicious use of antibiotics.

Keywords: VRE, Linezolid, Daptomycin, E test.

Introduction

Enterococcus is a genus of facultatively anaerobic, Gram-positive organisms of ovoid shape found in pairs or short chains. Previously, they were classified as Streptococcus Group D. [1] Nosocomial infections are often caused by Enterococci, which are known as opportunistic pathogens. *Enterococcus faecalis* and *Enterococcus faecium* are two of the most common *Enterococcus* species that are

associated with human diseases. Infections caused by them include bacteremia, endocarditis, urinary tract infections, surgical wound infections, and intra-abdominal and intra-pelvic infections. Vancomycin-resistant Enterococci have been on the rise over the last two decades. [2] There has been an increase in resistance to the most common anti-Enterococcal antibiotics, including ampicillin and aminoglycosides, and they are inherently resistant to many other antibiotics, such as cephalosporins and clindamycin, making these infections difficult to treat. [3]

Infections caused by *Enterococcus* can be treated with glycopeptide antibiotics. However, glycopeptide resistance is also on the rise. There are six types of glycopeptide resistance described in Enterococci, based on the sequence of the structural gene for the resistance ligase (vanA, vanB, vanC, vanD, vanE, and vanG). The VanA type of resistance is characterized by a high level of resistance to vancomycin and teicoplanin. In contrast, the VanB type is characterized by variable levels of resistance to vancomycin and teicoplanin. VanD strains are resistant to moderate levels of vancomycin and teicoplanin. VanC, VanE, and VanG isolates exhibit low-level resistance to vancomycin only. [4] Vancomycin resistance was found in 24% of *Enterococcus* isolates in a study by Phukan et al. [5] The first report of a linezolid-resistant *Enterococcus* in India came from Kolkata, but there have been very few reports since then. A G2576T mutation in domain V of 23S ribosomal ribonucleic acid (rRNA) genes of *Enterococcus* causes clinical resistance to linezolid. [6]

Both intrinsic and acquired resistance to many antimicrobials is known to exist in *Enterococcus* species. There are many resistance genes present that act against various antimicrobials, and this is the most common mechanism responsible for intrinsic resistance. The acquired resistance among Enterococci is caused by DNA mutation or by acquiring new genes through gene transfer. The result is the development of resistance to a variety of antibiotics, including vancomycin, tetracycline, macrolides, fluoroquinolones, etc. Multidrug-resistant isolates are those that are resistant to three or more antimicrobial classes. [7] There has been an increase in multidrug-resistant bacteria (MDR) in clinical and environmental specimens over the last 50 years. Multidrug-resistant organisms are also known as superbugs. Among the most dreaded multidrug-resistant organisms are Gram negative bacilli such as Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter spp. In contrast, Gram-positive bacteria such as Staphylococcus aureus and *Enterococcus faecium* have also been reported to display multidrug resistance. [8] To develop resistance to antimicrobials, bacteria have developed a variety of mechanisms. Resistance is caused by several mechanisms. The most significant of these is horizontal gene transfer. Biofilms are also produced by some bacteria. Biofilms remain adherent to the surface and help the bacteria to evade the attack of different antimicrobials. [9]

Emergence of vancomycin intermediate or resistant S. aureus has created the need for other anti‑MRSA antibiotics. Many alternatives for treatment of MRSA infection including linezolid and daptomycin are currently approved by Food and Drug Administration. However, the emergence of resistance to linezolid and daptomycin in MRSA isolates has been recently reported. [10,11] The aim of the present study was to measure the prevalence of vancomycin, linezolid and daptomycin resistance in *Enterococcus* clinical isolates from patients in a tertiary hospital.

Materials and Methods

This study was a retrospective study conducted by Department of Microbiology, AIIMS, Patna, Bihar, India .Total of 500 consecutive, non-repetitive clinical isolates of *Enterococcus* species from different clinical samples were included in the study. The study period was two years

Enterococcus species were isolated and identified in accordance with standard procedures. [12] Antibiotic susceptibility testing (ABST) of all isolate was conducted by both disc diffusion method and Minimum inhibitory concentration (MIC) on automated Vitek (BioMe´rieux) system. [13] *Enterococcus* ATCC strains E. faecalis ATCC 29212 were used as standards for antibiotic susceptibility testing. Strains resistant to vancomycin, linezolid and daptomycin were confirmed further tested by E test strip (Himedia, Mumbai). For automated ABST and E test manufacturer instructions were followed. Minimum inhibitory concentration (MIC) interpreted was as per criteria laid by Clinical Laboratory Standards Institute (CLSI) guidelines. [14]

Results

Table 1: Distribution of *Enterococcus* species in various clinical samples

Samples	Isolated (n/%)	Vancomycin (n)		Linezolid (n)		Daptomycin (n)	
		I	R	I	R	I	R
Urine	440 (88)	7	18	3	4	-	-
Blood	36 (7.20%)	1	-	-	-	-	-
Pus	12 (2.4%)	3	-	-	-	-	-
Sputum	8 (1.6%)	-	-	-	-	-	-
Others	4 (0.8%)	-	-	-	-	-	-
Total	500	11	18	3	-	-	-

A total of 500 *Enterococcus* were isolated during the study period. Of these, there were 350 (70%) isolates were of *Enterococcus faecalis* and 150 (30%) of *Enterococcus faecium*. No other *Enterococcus* species were isolated. Clinical samples included urine, blood, pus, sputum and other samples like body fluids etc. Out of these 500 isolates, the most common sample was urine (440 (88%)), followed by blood (36 (7.20%)), pus (12 (2.4%)), and sputum (8 (1.6%).

Table 2: MIC interpretative criteria and ABST pattern of *Enterococcus* isolates (µg/ml)

Organism	Vancomycin			Linezolid			Daptomycin		
	S	I	R	S	I	R	S	I	R
	≤4	8-16	≥32	≤2	4	≥8	≤1	2-4	≥8
Enterococcus faecalis	325	12	13	320	2	2	350	-	-
Enterococcus faecium	125	20	30	170	4	2	150	-	-

Among isolates of *Enterococcus*- 22, 43 and 435 were intermediate, resistant and sensitive to vancomycin respectively. Linezolid intermediate and resistant was identified in 2 and 4 isolates of *Enterococcus faecium* only. MIC to vancomycin ranged between 0.25-256 µg/ml, MIC of linezolid ranged between 0.25- 16µg/ml and MIC for daptomycin was less than 1 µg/ml for all the isolates.

Discussion

Vancomycin has been the cornerstone in the treatment of patients with serious methicillin‑resistant Staphylococcus aureus (MRSA) infections. Increased use of vancomycin has resulted in the emergence of MRSA with reduced susceptibility to vancomycin. [15-17] *Enterococcus* species constitutes normal

intestinal microflora in high proportion of healthy adults. *Enterococcus faecalis* and *Enterococcus faecium* are the two most common species of enterococci isolated from clinical samples. [18] E. faecalis are more prevalent than E. faecium, however increase in E. faecium clinical isolation have been reported recently. E. faecium infections are of clinical relevance, due to high percentage of resistant to vancomycin and ampicillin. [19]

In the hospitals, the real challenge in management of enterococcal infection lies in its intrinsic and acquired resistance to numerous antimicrobial agents. Researchers recommend combination therapy for high load enterococcal ampicillin for susceptible *Enterococcus* isolates and vancomycin for penicillin resistance isolates. However, many researcher have reported emergence of vancomycin resistance in *Enterococcus* species, which pose an immense challenge to the clinicians. [20] For vancomycin resistant clinical isolates, Infectious Diseases Society of America (IDSA) recommends linezolid or daptomycin antibiotics especially in bacteremia. [21] In views of these recommendation in vitro susceptibility testing for newer antimicrobials, such as daptomycin and linezolid, is essential for the management of VRE infections.

Six different types of vancomycin resistance are shown by *Enterococcus*: Van-A, Van-B, Van-C, Van-D, Van-E and Van-G. Van A confers high degree of resistance to both vancomycin (MIC 64µg/ml) and teicoplanin (MIC 16µg/ml), whereas Van B and Van E confers varying level resistance to vancomycin (MIC 4 - 1000 µg/ml), but are susceptible to teicoplanin. 18 Van A is the most common mechanism for resistance among clinical isolates, so identification of any intermediate susceptibility to vancomycin warrants detailed molecular investigation. In this study, Vancomycin intermediate susceptibility was noted among 11 isolates, which is in concordance with other studies. [22,23]

Linezolid resistance was detected in 4 *Enterococcus* isolates, whereas 3 isolates demonstrated intermediate susceptibility. Linezolid resistance in *Enterococcus* has been reported earlier by other researchers also. In this study, prevalence of linezolid resistance is relatively lower compare to other publisher report, due to robust hospital antibiotic policy. Further, in this study, two *Enterococcus* isolates demonstrated combined linezolid and vancomycin resistance. Combined resistance to both vancomycin and linezolid is very rare. These results are in concordance with findings reported by other researchers. [24,25] Daptomycin is lipopeptide antibiotic used in the treat-ment life-threatening infections caused by gram-positive organisms. Daptomycin activity in media requires presence of divalent cations, especially calcium ions. E- strips of daptomycin with supplemented calcium ions can be applied directly on Mueller Hinton Agar. In our study, calcium supplemented culture media was used for daptomycin activity and all isolates were sensitive to daptomycin. Daptomycin resistance in *Enterococcus* isolates, is rare and reported in cancer patients. [25,26] Researchers have reported association of daptomycin resistant in Enterococci species with earlier exposure to daptomycin and vancomycin resistance. Enterococcal strains with vancomycin hetero- resistance, may fail to respond to daptomycin therapy, despite in vitro susceptibility to daptomycin. [27]

Recommended therapy for serious enterococcal infections consists of synergistic combination of an aminoglycoside and a cell wall–active agent. However, many E. faecium isolates are intrinsically resistant to cell wall active antibiotics like penicillin and some acquires resistance to vancomycin. Further, this resistance is mediated by plasmids, which are easily transferable

between bacterial species. Newer antibiotics were developed for treatment Enterococci infections resistant to ampicillin, vancomycin, or the aminoglycosides. These antibiotics include linezolid, daptomycin, and tigecycline. However soon after clinical usage of linezolid, resistance to linezolid has also emerged, which is steadily rising. [28] Present recommendation for *Enterococcus* susceptible to ampicillin, but resistant to aminoglycosides is a combination of ampicillin plus daptomycin or linezolid. For *Enterococcus* isolates that are resistant to ampicillin and susceptible to aminoglycosides, aminoglycoside combined with vancomycin should be used. However, if the isolate is resistant to both ampicillin and aminoglycosides, management should include newer antibiotics daptomycin, linezolid, or vancomycin combined with another susceptible antimicrobial agent. [26,29,30]

Conclusion

Our study demonstrated relatively low prevalence of Vancomycin and Linezolid resistance among clinical isolates of *Enterococcus.* However, persistence of vancomycin pressure on hospital flora and emergence of isolates *Enterococcus* species with combined resistance to newer antibiotics, is a cause of concern. Linezolid and daptomycin are effective antibiotics against VRE. Strict implementation of hospital antibiotic policy with judicious use of antibiotics is a key to prevention of emergence of multidrug resistant strains of *Enterococcus* species.

References

1. Murray BE. The life and times of the *Enterococcus.* Clinical microbiology reviews. 1990 Jan;3(1):46-65.
2. Raza T, Ullah SR, Mehmood K, Andleeb S. Vancomycin resistant Enterococci: A brief review. J Pak Med Assoc. 2018 May 1;68(5):768-72.
3. Gupta V, Singla N, Behl P, Sahoo T, Chander J. Antimicrobial susceptibility pattern of vancomycin resistant enterococci to newer antimicrobial agents. The Indian Journal of Medical Research. 2015 Apr;141(4):483.
4. Depardieu F, Perichon B, Courvalin P. Detection of the van alphabet and identification of enterococci and staphylococci at the species level by multiplex PCR. Journal of Clinical Microbiology. 2004 Dec;42(12):5857-60.
5. Phukan C, Lahkar M, Ranotkar S, Saikia KK. Emergence of vanA gene among vancomycin-resistant enterococci in a tertiary care hospital of North-East India. The Indian Journal of Medical Research. 2016 Mar; 143(3):357.
6. 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 Jan;4(01):13-6.
7. Falagas ME, Karageorgopoulos DE. Pandrug resistance (PDR), extensive drug resistance (XDR), and multidrug resistance (MDR) among Gram-negative bacilli: need for international harmonization in terminology. Clinical infectious diseases. 2008 Apr 1;46(7): 1121-2.
8. Uruén C, Chopo-Escuin G, Tommassen J, Mainar-Jaime RC, Arenas J. Biofilms as promoters of bacterial antibiotic resistance and tolerance. Antibiotics. 2020 Dec 23; 10 (1):3.
9. Lebeaux D, Ghigo JM, Beloin C. Biofilm-related infections: bridging the gap between clinical management and fundamental aspects of recalcitrance toward antibiotics. Microbiology and Molecular Biology Reviews. 2014 Sep; 78(3):510-43.
10. Basireddy S, Singh M, Ali S, Kabra V. CFR gene mediated linezolid

resistance in staphylococcal isolates. Int J Pharm Bio Sci. 2014; 5:139-9.
11. Dortet L, Anguel N, Fortineau N, Richard C, Nordmann P. In vivo acquired daptomycin resistance during treatment of methicillin-resistant Staphylococcus aureus endocarditis. International Journal of Infectious Diseases. 2013 Nov 1;17(11):e1076-7.
12. Faron ML, Ledeboer NA, Buchan BW. Resistance Mechanisms, Epidemiology, and Approaches to Screening for Vancomycin- Resistant *Enterococcus* in the Health Care Setting. J Clin Microbiol. 2016;54 (10) :2436–47.
13. Ligozzi M, Bernini C, Bonora MG, De Fatima M, Zuliani J, Fontana R. Evaluation of the VITEK 2 system for identification and antimicrobial susceptibility testing of medically relevant gram-positive cocci. Journal of clinical microbiology. 2002 May; 40(5):1681-6.
14. Rasanen ME, Linna AM, Santos JCR, Negri FR. Late Miocene Tidal Deposits in the Amazonian Foreland Basin. Sci. 1995;269(5222):386– 90.
15. Niveditha N, Sujatha S. Worrisome trends in rising minimum inhibitory concentration values of antibiotics against methicillin resistant Staphylococcus aureus-Insights from a tertiary care center, South India. Brazilian Journal of Infectious Diseases. 2015 Nov; 19:585-9.
16. Dhand A, Sakoulas G. Reduced vancomycin susceptibility among clinical Staphylococcus aureus isolates ('the MIC Creep'): implications for therapy. F1000 medicine reports. 2012 ;4.
17. Chang W, Ma X, Gao P, Lv X, Lu H, Chen F. Vancomycin MIC creep in methicillin-resistant Staphylococcus aureus (MRSA) isolates from 2006 to 2010 in a hospital in China. Indian journal of medical microbiology. 2015 Apr 1;33(2):262-6.
18. Dziri R, Lozano C, Said LB, Bellaaj R, Boudabous A, Slama KB, Torres C, Klibi N. Multidrug-resistant enterococci in the hospital environment: detection of novel vancomycin-resistant E. faecium clone ST910. The Journal of Infection in Developing Countries. 2016 Aug 31;10 (08):799-806.
19. Prasad KN, Tripathi A, Shukla SK, Singh A. 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.
20. Iosifidis E, Evdoridou I, Agakidou E, Chochliourou E, Protonotariou E, Karakoula K, et al. Vancomycin-resistant *Enterococcus* outbreak in a neonatal intensive care unit: Epidemiology, molecular analysis and risk factors. Am J Infect Control. 2013; 41(10):857–61.
21. Pendleton JN, Gorman SP, Gilmore BF. Clinical relevance of the ESKAPE pathogens. Expert Rev Anti-infect Ther. 2013;11(3):297– 308.
22. Phukan C, Lahkar M, Ranotkar S, Saikia K. Emergence of vanA gene among vancomycin-resistant enterococci in a tertiary care hospital of North - East India. Indian J Med Res. 2016;143(3):357–61.
23. Song JY, Cheong HJ, Seo YB. Clinical and microbiological characteristics of vancomycin-resistant enterococci with the VanD phenotype and vanA genotype. Jpn J Infect Dis. 2013; 66(1):1–5.
24. de Almeida LM, de Araujo MRE, Iwasaki MF, Sacramento AG, Rocha D, da Silva LP, et al. Linezolid Resistance in Vancomycin-Resistant *Enterococcus faecalis* and *Enterococcus faecium* Isolates in a Brazilian Hospital. Antimicrob Agents Chemother. 2014;58(5):2993– 4.
25. Chacko KI, Sullivan MJ, Beckford C, Altman DR, Ciferri B, Pak TR, et al.

Genetic Basis of Emerging Vancomycin, Linezolid, and Daptomycin Heteroresistance in a Case of Persistent *Enterococcus faecium* Bacteremia. Antimicrob Agents Chemother. 2018;62(4):1–9.
26. Britt NS, Potter EM, Patel N, Steed ME. Comparison of the Effectiveness and Safety of Linezolid and Daptomycin in Vancomycin-Resistant Enterococcal Bloodstream Infection: A National Cohort Study of Veterans Affairs Patients. Clin Infect Dis. 2015; 61(6):871–8.
27. Arias CA, Torres HA, Singh KV, Panesso D, Moore J, Wanger A, et al. Failure of Daptomycin Monotherapy for Endocarditis Caused by an *Enterococcus faecium* Strain with Vancomycin-Resistant and Vancomycin Susceptible Subpopulations and Evidence of In Vivo Loss of the vanA Gene Cluster. Clin Infect Dis. 2007; 45(10):1343– 6.
28. Crank C, O'Driscoll T. Vancomycin-resistant enterococcal infections: epidemiology, clinical manifestations, and optimal management. Infect Drug Resist. 2015;8(1):217.
29. Humphries RM, Pollett S, Sakoulas G. A Current Perspective on Daptomycin for the Clinical Microbiologist. Clin Microbiol Rev. 2013;26(4):759–80.
30. Pérez A. D., Valle D. M., Medina L. C. G., Burgos R. A. O., Reyes J. D. S., Solano O. I. A., Anguila J. J. M., & Rojas M. F. R. Assisted Therapy with Vacuum and Floating Stoma: A New Way to Treat a Periostomal Abscess. Journal of Medical Research and Health Sciences, 2021; 4(12): 1629–1635.