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A STUDY OF ANTIMICROBIAL RESISTANCE PATTERN OF ENTEROCOCCI FROM CLINICAL SPECIMEN WITH SPECIAL REFERENCE TO GLYCOPEPTIDE ANTIBIOTICS IN TERTIARY CARE HOSPITAL

Dr Neha Komatwar[1], Dr Poonam Sayare[2], Dr Kavita Bhilkar[3], Dr Sabina Jahagirdar[4], Dr Chhaya Chande[5], Dr Ameeta Joshi[6]

[1]Junior resident, GGMC & Sir JJ Hospital Mumbai (First author)
[2]Assistant professor, GGMC & Sir JJ Hospital Mumbai
[3]Associate professor, GGMC & Sir JJ Hospital Mumbai
[4]Assistant professor, GGMC & Sir JJ Hospital Mumbai
[5]Professor GGMC & Sir JJ Hospital Mumbai
[6]Professor & Head , GGMC & Sir JJ Hospital Mumbai

Corresponding Author

Dr Kavita Bhilkar
Associate professor, GGMC & Sir JJ Hospital Mumbai

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ABSTRACT

Enterococci are nosocomial infections that are resistant to a wide variety of innate and acquired resistance determinants as well as all therapeutically available antibiotics. The clinical microbiology lab plays a crucial role in applying accurate, repeatable, and useful antimicrobial susceptibility testing methods to guide the appropriate treatment of patients. A prospective study was conducted in the Department of Microbiology, Grant Govt. Medical College and Sir JJ hospitals Mumbai Maharashtra India, over eighteen months (April 2021 to October 2023) after obtaining clearance from the Institutional Ethics Committee, with 250 Enterococci, isolated from various clinical specimens. The isolates were identified by morphological and biochemical tests,which were then evaluated for antimicrobial susceptibility to several antibiotics using the Kirby-Bauer disk diffusion method. The minimum inhibitory concentration (MIC) of vancomycin and teicoplanin was measured using agar dilution and micro broth dilution methods, respectively.Of the 250 species of Enterococcus that were isolated, 170 (68%), were E. faecalis, 79(31.6%)were E. faeciumand one isolate of E. durans (0.4%).30.8% were male and 69.2% were female. While77.6% came frominpatient specimens, 22% of the isolates came from OPD patient specimens.Urine (67.2%) was the most predominant sample. Gentamicin resistance was found in 47.2% of patients, as was streptomycin resistance in 46.8%. Among 74 (29.6%) VRE isolates, 89.18% were resistant to levofloxacin, followed by ciprofloxacin at 82.43%. Van A phenotype was found in 68 (91.89%) of the VRE isolates. Several studies have found an increase in infection rates and antibiotic resistance among Enterococcus species. High resistance to routinely used antibiotics has exacerbated the situation, as has the emergence of VRE strains. To lower the occurrence of VRE, we recommend adequate use of antibiotics and infection control measures in our healthcare settings.

Keywords: antimicrobial resistance, high level gentamicin, Vancomycin resistant enterococci.

INTRODUCTION

The most common gram positive cocci in the intestinal flora of humans and other animals have been associated with several clinical illnesses, including pelvic, intraabdominal, endocarditis, and urinary tract infections.[1] Enterococci play an important role in the development of nosocomial and community acquired infections, including nosocomial bacteremia, surgical wound infections, and urinary tract infections.[1,2] It can also cause liver abscesses, adult central nervous system infections, and, less commonly, osteomyelitis, lung infections, and meningitisininfants.[3,4] According to a CDC survey, enterococcus caused 13.9% of nosocomial infections.[5]Enterococci display two types of antimicrobial resistance: intrinsic resistance, which is species-specific, and acquired resistance, which is due to new DNA acquisition or DNA mutation.[6] The combination of gentamicin and penicillin for therapeutic purposes is no longer effective due to aminoglycoside resistance.7Recognizing Vancomycin Resistant Enterococci (VRE) colonization and infection needs the laboratory's ability to identify enterococci as well as detect vancomycin resistance rapidly andcorrectly.8The overall incidence in India is 12.4%, with E. faecalis being the most often isolated species (39.37%), followed by E. faecium (19.66%). Antibioticresistant enterococci, such as those resistant to vancomycin, aminoglycosides, and penicillin, pose

serious threat to healthcare institutions and medical professionals. Thus, the current study's objective is to determine the species and their antibiogram that are prevalent in our hospital environment.

MATERIALS AND METHODS

A prospective study was conducted in the Department of Microbiology, Grant Govt. Medical College and Sir JJ Hospitals Mumbai Maharashtra India, over a period of 18 months (April 2021 to October 2023) after obtaining clearance from the Institutional Ethics Committee.A total of 250enterococci isolates were included in this study. All the clinical samples (urine, pus, blood, body fluids) from patients of the Indoor and Out-patient Department, received by the Department during this period were processed by standard laboratory protocol[9] and isolated enterococcus were included in this study. Enterococcal isolates from the gastrointestinal tract, female genital tract, and oral cavity were excluded.

The genus Enterococcus was confirmed by Gram stain, i.e., gram-positive cocci in pairs and short chains, colony characters, pH, temperature, catalase test, and biochemical tests like bile esculin hydrolysis, salt tolerance test using 6.5% NaCl. Arginine decarboxylation and sugar fermentation served as the basis for the speciation. Following inoculation on Blood Agar and MacConkey's Agar, the samples were aerobically incubated for 24 hours at 37°C. Gram staining, motility, colony features, and standard microbiological procedures were used to identify enterococci. After the inoculum for antimicrobial susceptibility testing was standardized to 0.5 McFarland standards for different enterococci isolates by the CLSI guidelines, the Kirby–Bauer disc diffusion method was carried out.[10] Mueller-Hinton agar supplemented with 5% sheep blood was used.[11] The antibiotic discs used were as follows: ampicillin (10µg), penicillin (10µg), ciprofloxacin (5µg),tetracycline (30µg), erythromycin (15µg), vancomycin (30µg), nitrofurantoin (300µg), ceftriaxone (30µg), teicoplanin (30µg), and linezolid (30µg), high-level gentamicin (120µg), and highlevel streptomycin (300µg).The inoculated plates were incubated at 35°C for 18 hours. Using the CLSI criteria (2023), the diameter of each antibiotic's zone of inhibition was measured and interpreted as sensitive, intermediate sensitive, or resistant. Enterococcus faecalis ATCC 29212 and E. faecalis ATCC 51299 were used as the susceptible and resistant quality control strains.[12]Minimum inhibitory concentrations (MIC) of vancomycin were determined by the agar dilution method. The test organism was grown in broth and the turbidity matched with McFarland 0.5 standard (approximately 1.5 × 108 cfu/mL). Spot inoculation of the agar medium was done using 10 µl of bacterial culture. The plates were incubated at 37°C for 24 h and examined. The minimum concentration of vancomycin that inhibited bacterial growth was considered MIC. The MIC of teicoplanin was determined by the broth microdilution method.Through the examination of Teicoplanin and Vancomycin resistance patterns, various genotypes of the Van gene were analyzedand the findings were interpreted as per CLSI guidelines.[10]

RESULTS

A total of 250 Enterococcus species were isolated and identified, in the Microbiology laboratory, Department of Microbiology, Grant Govt. Medical College and Sir JJ Hospitals MumbaiMaharashtra India,over a period of eighteen months (April 2021 to October 2023). Among 250 Enterococcus species, 170(68%)species were Enterococcus faecalis, and 79(31.6%) species were Enterococcus faecium and a single isolate of E. durans. E. faecalis was the predominant species.

The highest prevalence of enterococcus was seen infemales 173(69.2%) followed bymales 77(30.8%), with M: F=1:2.Among 77 isolates of males, maximum isolates (25.97%) were found in the age group of 51-60 years. Out of the 173 isolates from female patients, the maximum isolates (39.30%) were found in the age group of 18-30 years.

194 isolates (77.6%), were from specimens collected from patients admitted in the hospital wards (in-patients) and 56(22%) isolates were from specimens collected from patients on an out-patient basis (OPD patients).Sample distribution of cases, maximum enterococcus 168(67.2%)isolated from urine specimenfollowed by pus (23.2%), blood (5.6%), and sterile body fluids (4%).

From antimicrobial susceptibility testing results, it is observed that 80.4% of enterococcal isolates were highly resistant to Tetracyclines (84%), Norfloxacin (80.4%) followed by levofloxacin (78.8%) and ciprofloxacin (78.4%). 79.03% of isolates were resistant to erythromycin while 73.68% of isolates were susceptible to linezolid and 62% were susceptible to rifampicin.

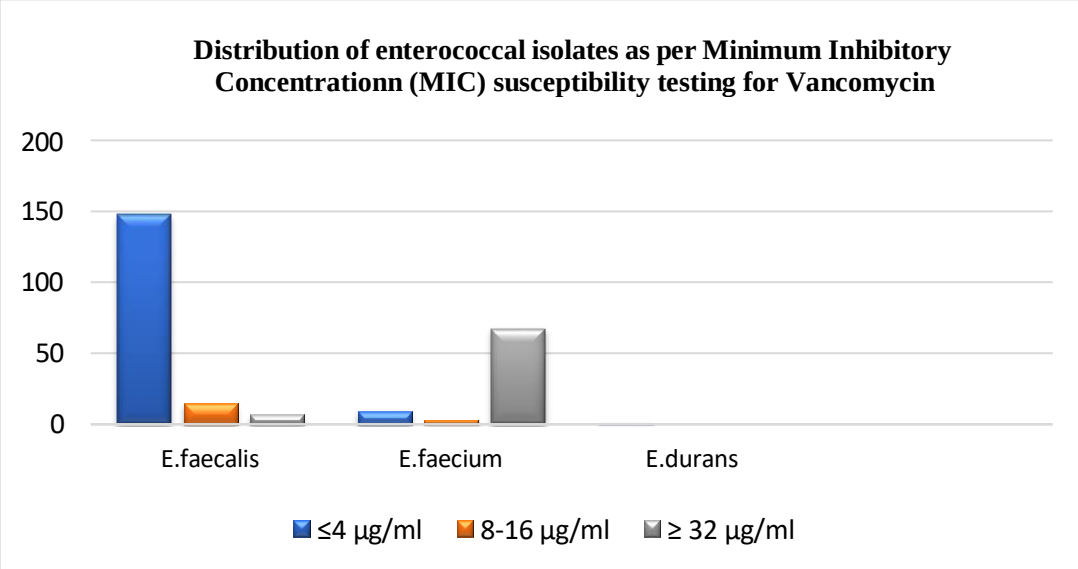
All the enterococcal isolates were subjected to test for High-level Aminoglycoside resistance by two methods named Gentamicin and Streptomycin high concentration disc diffusion method and MIC method.Urine was the most predominant high-level gentamicin resistance seen in 118 (47.2%) cases and high-levelstreptomycin resistance in 117(46.8%) cases.Out of 250 enterococcal isolates, 68.35% of E. faecalis showed high-level gentamicin resistance and 52.94% resistance to high level streptomycin.E. faecium showed 81.01% high level gentamicin resistance and 34.17% resistance for high level streptomycin. E. durans showed no resistance to either of the high-level aminoglycosides.

Out of 170 E.faecalis, 87.05% of isolates were susceptible to vancomycin with MIC ≤4 µg/mL. There were 8.82% E.faecalis isolates which showed an intermediate susceptible pattern with MIC 8 to 16 µg/mL. While 4.11% E.faecalis isolates were resistant with MIC ≥32 µg/mL. Out of 79 E.faecium isolates, there were 11.39% isolates susceptible to vancomycin with MIC ≤4 µg/mL. There were 3.79% E.faecium isolates which were intermediate susceptible with MIC 8

to 16 µg/mL. There were 84.81% E.faecium isolates resistant with MIC ≥32 µg/mL.E.durans showed 100 % susceptibility to vancomycin with MIC ≤4 µg/mL.

Table1: Minimum Inhibitory Concentration (MIC) breakpoint of enterococcal isolates for Vancomycin by agar dilution method:

erococcus species	No. of isolates	Susceptible (≤4 µg/mL)	ntermediate Susceptible (8-16µg/mL)	Resistant (≥32µg/mL)
E.faecalis	170	148(87.05%)	15(8.82%)	7(4.11%)
E.faecium	79	9(11.39%)	3(3.79%)	67(84.81%)
E.durans	1	1(100%)	-	-
Total	250	158	18	74



Out of 170 E.faecalis isolates there were 80% isolates susceptible to teicoplanin with MIC ≤8µg/mL. There were 7.64% E.faecalis isolates with intermediate susceptible with MIC 16µg/mL. While 12.35% E.faecalis isolates were resistant with MIC ≥32 µg/mL. Out of 79 E.faecium isolates, 87.34% isolates were resistant with MIC ≥32 µg/mL while 11.39% isolates were susceptible to teicoplanin with MIC ≤8µg/mL. There were 1.26% E.faecium isolates with intermediate susceptible pattern with MIC 16µg/mL.E.durans was susceptible to teicoplanin with MIC ≤8µg/mL.

Table 2. Minimum Inhibitory Concentration (MIC) breakpoint of enterococcal isolates for Teicoplanin by Micro broth dilution method:

erococcus species	No. of isolates	Susceptible (≤8µg/mL)	ntermediate Susceptible (16µg/mL)	Resistant (≥32µg/mL)
E.faecalis	170	136(80%)	13(7.64%)	21(12.35%)
E.faecium	79	9(11.39%)	1(1.26%)	69(87.34%)
E.durans	1	1(100%)	-	-
Total	250	146(58.4%)	14(5.6%)	90(36%)

Among 74 VRE isolates, 66 (89.18%) were resistant to levofloxacin followed by ciprofloxacin (82.43%), and norfloxacin (81.08%). 51(68.91%) VRE showed high level gentamicin resistance while only 23 (31.08%) were resistant to high level streptomycin. 91.89 % of VRE were resistant to teicoplanin.

VRE cases were detected in 74 (29.6%)isolates out of which VanA type (MIC values in the range of 64 to 256 µg/mL) was detected phenotypically in most cases (91.89%) followed by VanB (8.10%) (MIC values in the range of 64 to 128 µg/mL).

Table 3. Phenotypic distribution of enterococcal isolates

Phenotype	Species	Total isolate	MIC	
			Vancomycin (µg/mL)	Teicoplanin (µg/mL)
VanA (91.89%)	E.faecalis	8 (10.81%)	≥ 64(3)	≥ 32(2)
			≥ 128(1)	≥ 64(2)
	E. faecium	60 (81.08%)	≥ 64(23)	≥ 32(12)
			≥ 128(5)	≥ 64(15)
			≥ 256(2)	≥ 128(2)
				≥ 256(1)
VanB	E.faecalis	6 (8.10%)	≥ 64 (6)	≤4 (6)

DISCUSSION

The changing clinical patterns of the Enterococcus infections and their antimicrobial susceptibility patterns have become an important topic of discussion, as it is emerging as nosocomial pathogen nowa-days.[13]

In the present study, a maximum number of enterococcal isolates were found in the age group of 18-30 years (30%). Similar findings were observed in a study done by Srivastava P et al[14]in 2013 wherein a maximum number of cases (40%) were in the age group of 21-40 years.

In our study, 77.6% of the cases were in patients, who were admitted in various wards (Medical, surgical, obstetrics, paediatrics, and various ICUs like NICU, MICU, and PICU, and 22.4% were from the outpatient department. Similar distribution was observed in studies done by Jain S et al[15]in 2011, in which, 72% were from the inpatient department while 28% were from the outpatient department.

In the present study maximum number of isolates were from the urine samples (67.2%), similar to the study done by Ira Praharaj et al[16] in 2013 (59.2%).Also, 58 (23.2%) enterococci were isolated from pus samples. Similar results were seen by Golia S et al[17]in 2014 (19%) and Ira Praharaj et al[16] in 2013 (38%).Only 14 (5.6%) isolates were from blood in our study, while in a study by Mohanty S et al[18] in 2005 36.1% were blood samples.

In the present study, we isolated three species E.faecalis (68%), followed by E.faecium (31.6%), and E.durans (0.4%). The results are similar to the studies done by Rupali S Shinde et al[19] in 2012 where the most common species was E.faecalis (87.03%), followed by E.faecium (9.25%) and E.durans (3.7%)

In the present study, ampicillin resistance was high in E.faecium (33%), followed by E.faecalis (8%), which is similar to other studies done by Mendiratta DK et al[20] in 2004 where high resistance was seen in E.faecium (94.4%), followed by E.faecalis (82.7%). Adhikari L et al[21]in 2010 noted more resistance in E.faecium (27.69%), followed by E.faecalis (70.59%).

In the present study, there were 118 (47.2%) enterococcal isolates that showed a High-level of Gentamicin Resistance (HLGR) (120µg) and 117(46.8%) isolates that showed High-level Streptomycin Resistance (HLSR) (300 µg), which is similar to other studies done by Elango P et al., Padmaraj R et al[22] which showed 42.7% HLGR and 29.8%HLSR.

As per phenotypic classification, the majority of 68(91.89%) enterococcal isolates were found to have Van A phenotype. While the remaining 6 isolates were of Van B phenotype. A study done by Khanal LK et al[23] and Priyanka Paul Biswas et al[24]in 2023 also showed Van A phenotype to be the commonest followed by the Van B phenotype.

CONCLUSION

Speciation and regular monitoring for antibiotic resistance with special attention to vancomycin and high-level aminoglycosides is warranted. To reduce the VRE prevalence worldwide, appropriate use of antibiotics according to antimicrobial susceptibility testing should be encouraged. Efforts should be made to reduce the transmission of VRE isolates. Delayed identification of VRE carriers leads to an increase in nosocomial transmission of VRE.

REFERENCES

1. Rahangdale V.A. et al. Study of antimicrobial resistance in Enterococci. Indian Journal of Medical Microbiology 2008; 26(3): 285.
2. Sood S. et al. Enterococci infections and antimicrobial resistance. Indian Journal of Medical Research 2008; 128: 112.
3. Moellering RC. Emergence of Enterococci as a significant pathogen. J Clin Infect Dis 1992; 14:1173-1178.

4. Purva M, Rama C, Benu D, Nidhi S and Lalit K. Vancomycin-resistant Enterococcal bacteremia in a lymphoma patient. Indian J Med Microbiol1999; 17(4): 194-195
5. Huyele MM, Sahm DF, Gilmore MS. Multiple drug resistant Enterococci, the nature of the problem and an agenda for the future. Journal of Emerging Infectious Diseases 1998 June; 4(2): 1-10
6. Gold HS and Moellering RC. Antimicrobial drug resistance. N-Engl JMed 1996Nov;335(19): 1445-53.
7. Huycke MM, Sahm DF, and Gilmore MS. Multiple drug-resistant enterococci: The nature of the problem and an agenda for the future. Journal of Emerg Infect Dis 1998 June; 4(2): 1-10.
8. Mundy LM, Sahm DF, Gilmore M. Relationship between enterococcal virulence and antimicrobial resistance. Clin Microbiol Rev 2000 Oct; 13(4): 513-22.
9. Indian Council of Medical Research. Standard Operating Procedures Bacteriology. [18]2019;2(2):56-58. Winn, Washington C, and Elmer W. Koneman
10. Performance Standards for Antimicrobial susceptibility testing. 32nd Edition. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute;2023.
11. Wellinghausen N, Chatterjee I, Berger A, Niederfuehr A, Proctor RA, Kahl BC. [24] Characterization of clinical Enterococcus faecalis small-colony variants. J Clin Microbiol. 2009;47(9):2802-11.
12. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing, Twenty-First Informational Supplement (M100-S21). Vol. 31, No. 1. Wayne, Pennsylvania, USA: Clinical and Laboratory Standards Institute; 2011.
13. Desai PJ, Pandit D, Mathur M, Gogate A. The prevalence, identification and the [26] distribution of various species of Enterococci which were isolated from clinical samples, with special reference to the urinary tract infections in catheterized patients. India J Med Microbial.2001;19:132-37.
14. Abamecha A, Wondafrash B, Abdissa A. Antimicrobial resistance profile of Enterococcus species isolated from intestinal tracts of hospitalized patients in Jimma, Ethiopia. BMC Res Notes. 2015 Jun 3; 8:213.
15. Shinde RS, Koppikar GV, Seema Oommen S. Characterization and Antimicrobial susceptibility pattern of clinical isolates of Enterococci at a tertiary care hospital in Mumbai, India. Annals of Tropical Medicine and Public Health. 2012; 5(2):85-8.
16. Mathur P, Kapil A, Chandra R, Sharma P,Das B. Antimicrobial resistance in Enterococcus faecalis at a tertiary care center of northern India. Indian J Med Res. 2003; 118:25-8.
17. 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)
18. Mohanty S, Jose S, Singhal R, Sood S, Dhawan B, Das BK, et al. Species prevalence and antimicrobial susceptibility of enterococci isolated in a tertiary care hospital of North India. Southeast Asian J Trop Med Public Health. 2005; 36:962-5.
19. Shinde RS, Koppikar GV, Seema Oommen S. Characterization and Antimicrobial susceptibility pattern of clinical isolates of Enterococci at a tertiary care hospital in Mumbai, India. Annals of Tropical Medicine and Public Health. 2012; 5(2):85-8.
20. Mendiratta DK, Kaur H, Deotale V, Thamke DC, Narang R, Narang P. Status of high-level aminoglycoside resistant Enterococcus faecium and Enterococcus faecalis in a rural hospital of central India. Indian J Med Microbiol. 2008; 26(4): 369-71.
21. Adhikari L. High-level aminoglycoside resistance and reduced susceptibility to vancomycin in Nosocomial Enterococci.J Glob Infect Dis. 2010; 2(3):231-5.
22. Elango P, Padmaraj R,Srivani SR. High-level aminoglycoside resistant genes among clinical isolates of Enterococcus species in Chennai, India. Scientific World Journal 2014; 2014: 329157.
23. International Journal of Health Sciences & Research Phenotypic and Genotypic Characterisation of Vancomycin-Resistant Enterococci (VRE) in a Tertiary Care Hospital of Kanpur, India (www.ijhsr.org) 56 Vol.9; Issue: 5; May 2019
24. Endtz, H. P., N. Van Den Braak, A. Van Belkum, Wil. H. Goessens, D. Kreft, A. B. Stroebel, and H. A. Verbrugh. 1998. Comparison of eight methods to detect vancomycin resistance in enterococci. J. Clin. Microbiol.36:592–594