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Original Research Article

STUDY ON HIGH-LEVEL AMINOGLYCOSIDE AND VANCOMYCIN RESISTANCE AMONG ENTEROCOCCUS ISOLATES IN A TERTIARY CARE HOSPITAL

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ABSTRACT

Background: Enterococci are now recognized as significant nosocomial pathogens and one of the emerging threats in healthcare settings. These are inherently resistant to common antibiotics such as beta-lactams, cephalosporins, and lincosamides. Emergence of vancomycin-resistant enterococci and high-level aminoglycoside-resistant strains have further complicated the challenge leading to substantial threat to public health. **Materials and Methods:** A prospective study was conducted for a period of one year (April 2024 to March 2025) in the Department of Microbiology, I Care Institute of Medical Sciences And Research, Haldia, West Bengal, India. A total of 178 isolates of Enterococcus spp. from various clinical specimens were collected. The samples were subjected to gram stain and inoculated in Macconkey and blood agar. The isolates were identified by using various biochemical tests. Antimicrobial susceptibility testing was done as per CLSI guidelines (M100, 34th edition). Vancomycin resistant enterococci (VRE) were tested on Brain Heart Infusion agar supplemented with 6 µg /ml of vancomycin. High-level aminoglycoside resistance (HLAR) were tested by disc diffusion method using high-level gentamicin (120 µg) and high-level streptomycin (300 µg) discs, and further was confirmed by agar dilution method. **Result:** A total of 178 Enterococcus isolates were obtained in the study, majority of sample source was urine (58.4%). Out of 178 isolates, 71 (39.9%) were resistant to high-level aminoglycosides (HLAR), out of which 33 (18.5 %) were resistant to high-level gentamicin (HLGR), 29 (16.3%) were resistant to high-level streptomycin (HLSR) and 9 (5.1%) isolates were resistant to both high-level gentamicin and streptomycin. **Conclusion:** HLAR poses major therapeutic challenge among patients. Continuous surveillance, rational antibiotic use, and strict infection control practices are essential to limit the spread of these multidrug-resistant pathogens.

INTRODUCTION

1 Enterococcus is an aerobic and facultatively anaerobic gram positive cocci with ubiquitous occurrence. 2 They can not only be found inhabiting the mucosal surfaces of humans and animals as part of the commensal flora, they can also be found in plants, soil, water and even dairy products.[1,2,3,4] 3 Previously regarded as organisms with low pathogenicity, Enterococcus spp has now been regarded as one of the emerging threats in healthcare settings. 4 The ability of Enteroccoccus spp.to colonise the mucosal surfaces, combined with its capability for nosocomial spread,[5] and its intrinsic resistance to several antibiotics and emergence of multidrug resistant strains has made it a dreaded pathogen to deal with.[6,7] 5 The threat posed by the multidrug resistant strains of bacteria was highlighted in a study conducted by Louis B. Rice in 2008,[8] where Enterococcus faecium was included in the ESKAPE pathogens list, which was also included in the World Health Organisation's 'high' priority pathogen list in 2017.[9,10]

6 The spectrum of diseases caused by the various Enterococcus species ranges from urinary tract infections, bacteremia, particularly in immunocompromised patients, diabetic and decubitus ulcers, surgical site infections, peritonitis, gingival infections and even root canal failure.[11]

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7 Enterococcus exhibits intrinsic resistance to most cephalosporins, clindamycin, trimethoprim-sulfamethoxazole and low level resistance to aminoglycosides like gentamicin and streptomycin. 8 Monotherapy in such cases can lead to treatment failures, which is overcome by the synergistic effect of combining a cell wall active agent like ampicillin, vancomycin and an aminoglycoside, which has been the mainstay of treatment for systemic enterococcal infections.[12] 9 But several studies have shown Enterococcus to be capable of exhibiting high-level resistance to aminoglycosides (HLAR) by acquiring genes encoding aminoglycoside-modifying enzymes.[13] 10 Glycopeptides like vancomycin and oxazolidinones like linezolid can be used for treatment of such cases.11 Emergence of vancomycin resistant Enterococcus (VRE) as a nosocomical pathogen poses even a larger threat in terms of limited treatment option, increased length of hospital stay and healthcare cost burden.[14,15]

12 Due to lack of appropriate data in regards to HLAR and VRE in our institution, this study was undertaken to estimate the prevalence of vancomycin resistance and high-level aminoglycoside resistance in enterococcus isolates.

MATERIALS AND METHODS

13 This is a prospective study conducted for a period of one year (April 2024 to March 2025) in the Department of Microbiology, I Care Institute of Medical Sciences And Research, Haldia. 14 A total of 178 isolates of Enterococcus spp. were obtained from various clinical specimens like urine, pus, blood and other body fluids. 15 The samples were inoculated on appropriate culture media following standard procedures.16 Enterococcus species were identified by Gram's staining and biochemical tests like catalase test, bile esculin hydrolysis test, 6.5% NaCl test, sugar fermentation test and PYRase test.

17 Antimicrobial susceptibility testing was performed on cation adjusted Mueller-Hinton agar by Kirby-Bauer's disc-diffusion technique according to CLSI guidelines (M100, 34th edition).[16] 18 For antimicrobial susceptibility testing, readymade antibiotic discs (Hi-Media Laboratories, India) were used. 19 Penicillin (10 units), ampicillin (10 µg), doxycycline (30 µg), vancomycin (30 µg) and linezolid (30 µg) were tested. For urinary isolates, nitrofurantoin (300 µg), ciprofloxacin (5 µg) and levofloxacin (5 µg) were tested.[16] 20 High-level aminoglycoside resistance (HLAR) were tested by disc diffusion method using high-level gentamicin (120 µg) and high-level streptomycin (300 µg) discs. 21 A 6 mm zone of inhibition was resistant, between 7-9 mm inconclusive and zone size ≥ 10 mm considered susceptible. 22 Inclusive results were corroborated by agar dilution testing, where presence of more than 1 colony was considered resistant. 23 Vancomycin resistant enterococci (VRE) were tested on vancomycin screen agar (Brain Heart Infusion agar supplemented with 6 µg /ml of vancomycin), where growth of > 1 colony was presumptive of vancomycin resistance. Enterococcus faecalis ATCC 29212 was used as the standard reference strain.

24 The results obtained were analysed using Microsoft Excel 2010 software. 25 Statistical analysis was performed and a value of $p<0.05$ was considered statistically significant.

RESULTS

26 Among the 178 non repetitive isolates, 94 (52.8%) were identified as Enterococcus faecalis, 67(37.6%) identified as Enterococcus faecium and 17 (9.6%) belonged to other Enterococcus species. 27 Out of the 178 isolates of Enterococcus from the various clinical specimens, majority i.e 104 (58.4%) were isolated from urine, 47 (26.4%) from pus (including wound swabs) followed by 10 (5.6%) from blood. 28 The other samples include 9 (5.1%) from root canal, 6 (3.4%) were corneal scrapings and 2 (1.1%) from body fluids (1 asctic and 1 peritoneal fluid). 29 The sample wise distribution of the different Enterococcus species is depicted in Table 1.

30 Among the various departments, 47(26.4%) were from the various Intensive care units(ICUs), 41(23%) from various Out Patient Departments(OPD) and the rest were from various wards including 26 (14.6%) from surgery, 24 (13.5%) from obstetrics and gynaecology, 21 (11.8%) from medicine and 19 (10.7%) from paediatrics as shown in Fig. 1.

31 All the isolates (178) were sensitive to linezolid (100%), followed by vancomycin 157, ampicillin 65.7% 117, benzylpenicillin 61.2% 109 and doxycycline 54.5% (97). Nitrofurantoin, ciprofloxacin and levofloxacin were tested only for urine isolates as per CLSI M100 (34th ed) guidelines. 32 Out of 104 urinary isolates, 82 (78.8%) isolates were sensitive to nitrofurantoin, followed by 60 (57.7%) isolates of levofloxacin and 49(47.1%) isolates of ciprofloxacin. 33 The sensitivity pattern of the different Enterococcal isolates is shown in Figure 2 and Figure 3.

34 From a total of 178 isolates, 71 (39.9%) were resistant to high-level aminoglycosides (HLAR), out of which 33 (18.5 %) were resistant to high-level gentamicin (HLGR), 29 (16.3%) were resistant to high-level streptomycin (HLSR) and 9 (5.1%) isolates were resistant to both high-level gentamicin and streptomycin. 35 Out of the 33 high-level gentamicin resistant isolates, 16 (48.5%) were Enterococcus faecium, 11 (33.3%) were Enterococcus faecalis and 6 (18.2%) isolates of other Enterococcus species. 36 Among the 30 high-level streptomycin resistant isolates, 18(60%) were Enterococcus faecalis, while the rest 12(40%) were Enterococcus faecium. 37 7 isolates of Enterococcus faecalis and 5 isolates of Enterococcus faecium were resistant to both high-level aminoglycosides (gentamicin and streptomycin), which is depicted in Figure 4. 38 Most of the high-level aminoglycoside

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resistant Enterococci were isolated from urine (35/71) followed by pus (27/71), blood (15/71).

39 Out of 178 isolates of Enterococcus, 21(11.8%) were resistant to vancomycin. 40 Among the 21 isolates, 9 (42.9%) were E. faecium, 8 (38.1%) were E. faecalis and 4 (19.1%) isolates belonged to other Enterococus species. 41 Among these 21 VRE isolates, 9 (42.9%) were isolated from urine, 7(33.3%) from pus, 3(14.3%) from blood and 2 (9.5%) were root canal samples. 42 Among 21 VRE isolates, 14(66.7%) isolates (9 E. faecium, 4 E. faecalis, 1 Enterococcus spp) also showed resistance to high-level aminoglycosides. 43 Out of these 14, 8 isolates were resistant to vancomycin and high-level gentamicin while 6 isolates were resistant to vancomycin and high-level streptomycin as depicted in Table 3. 44 Among the 14 isolates resistant to both vancomycin and high-level aminoglycosides, 7 were from pus, 4 from urine and 3 from blood. 45 Sample wise distribution of HLAR and VRE isolates is shown in Table 4, where p=0.54, which is not statistically significant.

Table 1: Distribution of different Enterococcus species isolated from various clinical samples.

Enterococcus species(n=178)	Urine (n=104)	Pus+wound swab(n=47)	Blood (n=10)	Root canal (n=9)	Corneal scraping (n=6)	Body fluid (ascitic, pleural)n=2
E. faecalis (n=94)	52	23	5	7	5	2
E. faecium (n=67)	44	18	2	2	1	0
Other Enterococcus spp.(n=17)	8	6	3	0	0	0

Table 2: High-level aminoglycoside resistance pattern among VRE isolates

HLAR	No. of isolates	%
HLGR	8	57.1
HLSR	6	42.9

HLSR: High-level streptomycin resistance HLGR: High-level gentamicin resistance
HLAR: High-level aminoglycoside resistance VRE: Vancomycin resistant Enterococci

Table 3: Sample wise distribution o high-level aminoglycoside and vancomycin resistant isolates

Type of resistance	Urine	Pus	Blood	Root-canal
HLAR(n=71)	35	27	15	-
VRE (n=21)	9	7	3	2
VRE+HLAR(n=14)	4	7	3	-

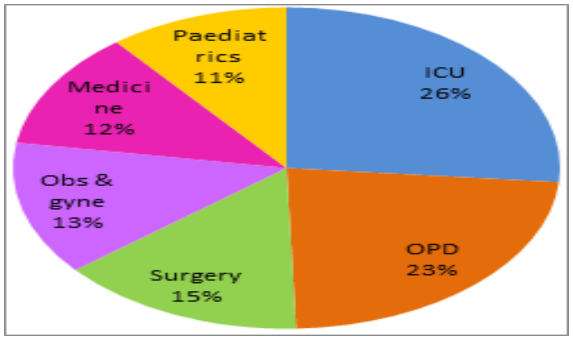

Figure 1: Distribution of Enterococcus isolates according to source

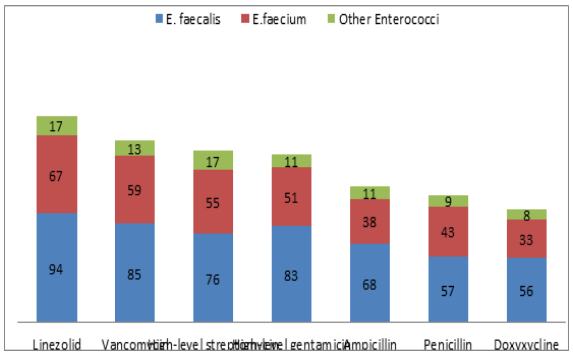

Figure 2: Antibiotic sensitivity pattern of different Enterococcal isolates

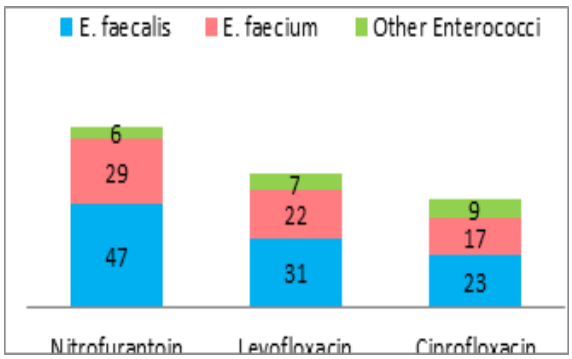

Figure 3: Sensitivity pattern of antibiotics for urine isolates

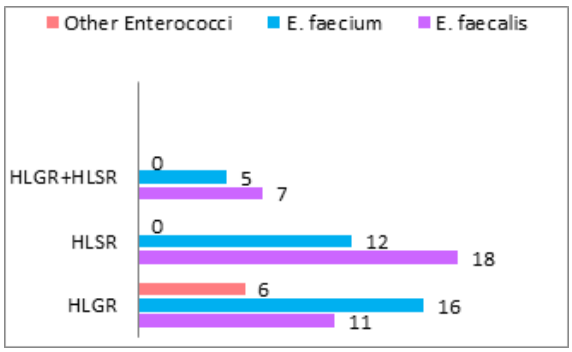

Figure 4: Distribution of different high-level aminoglycoside resistant isolates according to species

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HLSR: High-level streptomycin resistance HLGR: High-level gentamicin resistance.

DISCUSSION

46 Enterococcus has several species, among which Enterococcus faecalis and Enterococcus faecium are most commonly implicated in human infections.[17] 47 Among the other species of Enterococcus, E. casseliflavus, E. gallinarum ,E. durans, E. raffinosus are also common in healthcare settings.[18]

48 In our study, out of 178 Enterococcal isolates, 94 (52.8%) were Enterococcus faecalis and 67(37.6%) identified as Enterococcus faecium, while the other Enterococcus species while 17 (9.6%) isolates belonged to other Enterococcus species. 49 This is similar to the findings of Diab et al.(2019),[19] where E. faecalis and E.faecium were the most common isolates comprising of 56.7% and 30% respectively , while in a study by Yangzom T and Kumar Singh TS (2019),[20] E. faecalis and E.faecium were 68.7% and 20.9% while other species comprised of 10.4%. 50 Most of the isolates in our study were obtained from urine (58.4%), pus (26.4%) and blood (5.6%), followed by other clinical samples like root canal (5.1%), corneal scrapings (3.4%) and 1.1% from body fluids (asctic and peritoneal fluid). 51 This is in accordance with the findings of Kaarthiga S et al.(2020),[21] where urine was the most common clinical sample (68%) followed by pus (16%) and blood (14%). 52 In a study by Hota S et al.(2024),[22] urine was the most common clinical sample (76%) followed by blood (9%), pus (6%) and other samples (9%), while in a study by Arundathi et al.(2022),[23] pus (42.2%) was the most common sample followed by urine (32.8%) and blood (25%).

53 Most of the Enterococcal isolates were obtained from indoor patients (50.3%) including the different ICUs (26.4%) comprising a total of 137 (77%) out of 178 isolates, while only 41 (23%) isolates were obtained from the various out- patient departments including medicine, surgery, obstetrics and gynaecology, paediatrics, ophthalmology, otorhinolaryngology, dentistry etc. 54 Similar findings were noted in a study by Hota S. et al.(2024),[22] and Mittal S. et al(2016),[24] where most of the Enterococci were isolated from in-patient departments (94.2% and 60% respectively) as compared to various out- patient departments(5.8% and 40% respectively). 55 The high isolation rate of Enterococcus species from the different ICUs can be attributed to its propensity for colonisation and nosocomial spread,[5] indicating a need for stricter infection control measures.

56 Overall antimicrobial susceptibility testing showed 100% sensitivity to linezolid, vancomycin (88.2%), nitrofurantoin (79.2%), ampicillin (71.3%), benzyl penicillin(61.2%), levofloxacin (60.6%), ciprofloxacin (57.7%) and doxycycline (54.5%). Several studies by Mittal S. et al.(2016), Rajesh S et al. (2017), Paul M et al.(2019) ,Yangzom T and Kumar Singh TS (2019), and Arundathi et al. (2022),[24,25,26,20,23] showed maxiumum sensitivity to linezolid (99%,98%, 100%,,99.5%,100% respectively) and vancomycin (95%,94%, 86%, 86.3%, 100% respectively). 57 In our study levofloxacin (57.7%.), doxycycline (54.5%) and ciprofloxacin (47.1%) were the least sensitive for which is similar to study by Arundathi et al. (2022),[23] where ciprofloxacin and levofloxacin showed 52% and 48% sensitivity. 58 Whereas, in another study by Paul M et al.(2019),[26] and Raina D et al.(2022),[27] showed maximum resistance to quinpristin-dalfopristin (89.2%) and benzyl penicillin(75%) respectively.

59 In our study, the overall prevalence of high-level aminoglycoside resistance is 39.9%, with 18.5% high-level gentamicin resistance, 16.3 % high-level streptomycin resistance and 5.1% isolate resistant to both. 60 Similar findings were seen in a study by Rajesh S et al.(2017),[25] where the prevalence of HLGR was 8%, HLSR was 4% and 2% of isolates were resistant to both. 61 Study by Yangzom T and Kumar Singh TS (2019),[20] show similar findings with reference to high-level streptomycin resistance which accounted for 26.9%. 62 But other studies showed higher prevalence of aminoglycoside resistance. 63 Studies by Kaarthiga S et al.(2020), Rajan R et al.(2021) and Arundathi et al. (2022),[21,27,23] showed 69% ,72.7% and 42% high-level gentamicin resistance respectively. 64 The lower prevalence of resistance to high-level aminoglycosides in our institute can be attributed to the judicious use of such antibiotics. 65 Enterococcus faecium showed 48.5% resistance to high-level gentamicin followed by E. faecalis (33.3%) and 40% resistance to high-level streptomycin. 66 Studies by Arundathi et al. (2022),[23] and Hota S et al.(2024),[22] also showed high resistance pattern in Enterococcus faecium.

67 In our study, 11.8% of all Enterococcal isolates showed resistance against vancomycin which was tested on vancomycin screen agar, which corroborates to findings by Rajesh S et al. (2017),[25] and Kaarthiga S et al.(2020),[21] showing 13.7% and 9.4% of VRE. 68 In other studies by Paul M et al.(2019),[26] and Raina D et al.(2022),[27] the prevalence of vancomycin resistant Enterococci ranges from to 0% to 22%. 69 Majority of the VRE isolates were E. faecium (42.9%), followed by E. faecalis (38.1%) and other species(19.1%), similar to the findings of Yangzom T and Kumar Singh TS (2019),[20] and Hota S et al.(2024).[22]

70 Among the VRE isolates, 66.7% showed resistance to high-level aminoglycosides as well, which is in accordance with findings of Yangzom T and Kumar Singh TS (2019),[20] and Raina D et al.(2022).[27]

71 In our study, we have detected vancomycin resistant strains on vancomycin screen agar. 72 Confirmatory testing methods like agar dilution technique or microbroth dilution technique were not performed. 73 Species differentiation of

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Enterococcus other than E. faecalis and E. faecium were also not performed, which would have helped in choosing the appropriate antimicrobial for treatment, as certain species like E. casseliflavus and E.gallinarum are intrinsically multi-drug resistant.
74 Despite the above mentioned limitations, data in regards to sample type, source, prevalence of high-level aminoglycoside and vancomycin resistant Enterococci were obtained. 75 The study findings will be helpful in framing hospital antimicrobial policy and improving patient outcomes.

CONCLUSION

76 The emergence of multidrug resistant strains of Enterococcus, particularly with the rise in vancomycin resistance, poses a challenge to the clinicians. 77 This study shows a significant percentage of high-level aminoglycoside resistance, while the only silver lining being a relatively lower percentage of vancomycin resistant Enterococci. 78 This shows that there is a need for stringent antimicrobial stewardship practices to foster judicious use of antibiotics, particularly vancomycin and linezolid. 79 To conclude, it can be said that the hospital infection control practices need to be strengthened in order to prevent the nosocomial spread of Enterococcus spp, including frequent screening for vancomycin resistant Enterococci.

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