ORIGINAL RESEARCH

Analysis of susceptibility pattern of Enterococcus species from Urinary tract infections with special reference to High level Aminoglycosides and Vancomycin

[1]Dr. Parasmita Das Choudhury, [2]Dr. Kalpana Bezborah

[1]Assistant Professor, Department of Microbiology, Gauhati Medical College and Hospital, Guwahati, Assam, India
[2](Retired) Professor & Head, Department of Microbiology, Tezpur Medical College and Hospital, Tezpur, Assam, India

Corresponding Author
Dr. Parasmita Das Choudhury
Assistant Professor, Department of Microbiology, Gauhati Medical College and Hospital, Guwahati, Assam, India
Email: parasmitadas@gmail.com

Received: 16 April, 2023 Accepted: 19 May, 2023

ABSTRACT

Background: Enterococci are one of the important causes of nosocomial urinary tract infections. The emergence of High-level Aminoglycoside Resistance (HLAR) and Vancomycin-Resistant Enterococci (VRE), have complicated the available treatment modalities for enterococci worldwide. **Objectives:** To determine the prevalence of Enterococcus species causing urinary tract infections and their antimicrobial susceptibility pattern in a tertiary care centre with special emphasis on high level aminoglycosides and vancomycin resistance. **Methods:** A total of 2405 urine samples were processed by standard protocol. All Enterococci identified, were further speciated and the antibiogram was determined by Kirby-Bauer disk diffusion method as per CLSIguidelines. Minimum inhibitory concentration (MIC) for vancomycin was determined by the Epsilometer test. Data were analyzed using SPSS version 20 and $p \leq 0.05$ was considered as significant. **Results:** Of 104 (4.53%) enterococcal isolates, 48 (46.1%) were identified as *Enterococcus faecalis*, 42 (40.4%) as *Enterococcus faecium* and 14 (13.5%) as *Enterococcus avium.* Overall, antibiogram of the isolates showed high resistance to norfloxacin (79.8%), ciprofloxacin (77.9%) and levofloxacin (74%). HLAR was observed in 53 (51%) isolates, significantly higher in *E. faecium* than *E. faecalis* (p=0.008). High-level Gentamicin resistance (HLGR) was seen in 41.3% cases and high-level Streptomycin resistance (HLSR) was seen in 34.6%. Occurrence of VRE in our setting was 1.9%. Multidrug resistance (MDR) was observed in 25.9% isolates. **Conclusions:** The study highlights the alarming rise in prevalence of MDR enterococci, especially to high-level aminoglycoside, which warrants immediate, adequate, and efficient surveillance program to prevent and control its spread.

Keywords: *Enterococcus faecalis*, *Enterococcus faecium*, High-level Aminoglycoside Resistance (HLAR), High level Gentamicin resistance (HLGR), High level Streptomycin resistance (HLSR), Urinary tract infection (UTI), Vancomycin-resistant enterococci (VRE)

INTRODUCTION

Urinary tract infections (UTIs) are one of the most frequent infectious diseases worldwide and the burden of UTIs is a substantial global health problem as approximately 150 million patients are diagnosed worldwide each year.[1] Enterococci, normally considered commensal members of healthy intestinal microbiota of humans and animals, have gained widespread importance due to their increased isolation rates in both community-related and nosocomial infections with substantial morbidity and mortality. Enterococci are most common cause of UTIs, followed by intra-abdominal, intra-pelvic abscesses or post-surgery wound infections and blood stream infections. Enterococcal UTIs are more likely to be acquired in hospital or long-term care settings, and thus, are more likely to be resistant to many antibiotics. In the ICU setting, enterococci cause almost 15% of healthcare-associated urinary tract infections.Of more than 50 species known,

Enterococcus faecalis and *Enterococcus faecium* together account for the majority of clinical isolates (*E. faecalis* 80–90% and *E. faecium*5-10%). Other less commonly isolated species causing human infections include *E. gallinarum*, *E. casseliflavus*, *E.avium*, *E.durans*, *E. raffinosus*, *E. mundtii*, and *E hirae*. [2,3]

Enterococcus species possesses a major therapeutic challenge because of having both intrinsic and acquired resistance to various antibiotics. Enterococci can develop resistance to aminoglycoside by two different mechanisms, one is the low-level resistance which is due to reduced cell wall permeability and this type can be overcome by using a combination of aminoglycoside and cell-wall-acting agents. Another mechanism is the high-level resistance (HLR) which is due to the production of aminoglycoside-modifying enzymes (AMEs). This enzyme in enterococci negates the synergistic activity of aminoglycoside when it is being combined with a cell-wall-acting agent.Enterococci often acquire antibiotic resistance through exchange of resistance encoding genes carried on conjugative transposons, pheromone-responsive plasmids, and other broad host range plasmids.[4,5]The inherent antibiotic resistance and dissemination of resistance genes has led to the emergence of multidrug-resistant (MDR) giving rise to high-level aminoglycoside-resistant (HLAR) and glycopeptide-resistant enterococci (GRE), including vancomycin-resistant enterococci (VRE).[5,6]Even though VRE were first reported in 1986, from the UK and France, in recent years, they have been found to be disseminated all around the world. Infections caused by VRE were found to be associated with adverse outcome such as extended length of hospital stay, increased cost and increased mortality. VRE leaves fewer therapeutic options and there is also potential risk of vancomycin resistance gene transfer between *Enterococcus* species and from Enterococci to other bacteria (*Staphylococcus aureus*).[7]Though the rate of isolation of VRE is currently not very high in India when compared to other countries (USA and Europe), still it is definitely on an increasing side.[8] The epidemiology of nosocomial VRE bacteraemia has been quite extensively studied [9] but studies on problems posed by VRE as pathogens in UTI are very few.[10]

The resistance to currently available antibiotics, leads to limited treatment options and results in natural selection and spread of MDR enterococci in the hospital environment. Many studies have been conducted in India to find out the magnitude of resistance in Enterococci isolated from clinical specimens, but very less work has been done in this region of North East India. In Assam, there is a lack of centralized, local surveillance data on the prevalence of UTIs and, furthermore, there is a paucity of comprehensive data regarding Multidrug resistant Enterococci causing UTIs. Therefore, the present study was undertaken to determine the prevalence of Enterococcus species causing urinary tract infections and their antimicrobial susceptibility pattern in a tertiary care centre with special emphasis on high level aminoglycosides and vancomycin resistance.

METHODS

STUDY DESIGN AND POPULATION

This was a hospital based cross-sectional observational study conducted in the Microbiology of Department, Tezpur Medical College and Hospital, a tertiary care hospital located in Assam, India for a period of one year. The study group included patients suspected or diagnosed with UTI and those who had at least one of the following clinical signs and symptoms: fever; burning and painful urination; the frequent urge to urinate; and oliguria with no other recognized cause. Clean catch mid-stream urine samples and catheterized urine samples were collected in sterile wide-mouthed containers using proper aseptic measures from both hospitalised and outdoor patients. In infants, urine was collected by aseptic subrapubic aspiration. The study protocol was approved by the Institutional Ethical Committee (IEC No:05/TMCH).

ISOLATION AND IDENTIFICATION OF *ENTEROCOCCUS* SPECIES

The samples were immediately processed and inoculated on Blood agar, MacConkey agar, Cysteine Lysine Electrolyte Deficient (CLED) agar using a calibrated loop and the plates incubated aerobically overnight at 37◦C. Urine cultures with significant bacteriuria ($\geq 10^5$ colony forming unit/ml) were further processed to identify Enterococci. Enterococcus species were identified by standard procedures. [11,12]

ANTIBIOTIC SUSCEPTIBILITY TESTING

Antimicrobial susceptibility testing was done on Muller Hinton agar by Kirby Bauer disk diffusion method as per Clinical Laboratory Standard Institute (CLSI) guidelines [13] using commercially available antibiotic discs (HiMedia, Mumbai, India). The antibiotics tested were Ampicillin (10µg), Penicillin (10µg), Doxycycline (30µg), Ciprofloxacin (5µg), Levofloxacin (5µg), Nitrofurantoin (300µg), Norfloxacin (10µg), Vancomycin (30µg) and Linezolid (3 µg) and results were interpreted as per CLSI guidelines. *Staphylococcus aureus* ATCC 25923 and *E. faecalis* ATCC 29212 were included as quality control strains.

DETECTION OF HIGH-LEVEL AMINOGLYCOSIDE RESISTANCE (HLAR)

Isolates were tested for HLAR by standard disc diffusion method as per CLSI guidelines using high content gentamicin (120µg) and high content streptomycin (300µg) discs. Observation of no zone or a zone diameter of ≤ 6mm was considered as

resistant, 7-9 mm as intermediate and ≥10mm as sensitive.

MIC FOR VANCOMYCIN-RESISTANT ENTEROCOCCUS

Minimum inhibitory concentration (MIC) value for vancomycin was determined using the Epsilometer test (E-test strip by Hi-media, Mumbai). Any Enterococcus was considered VRE if the MIC was ≥ 16µg/ml.

STATISTICAL ANALYSIS

Data was entered and analysed using Statistical Package for Social Sciences (SPSS) version 20 (IBM, USA). Comparison for categorical variables was calculated using chi-square test and p value <0.05 were considered statistically significant.

RESULTS

Of the 2405 urine samples screened for UTI, 2293 specimens yielded growth and 104 (4.53%) samples were identified as Enterococci during the study period. 48 (46.1%) were identified as *E. faecalis*, 42 (40.4%) as *E. faecium* and the remaining 14 (13.5%) as *E. avium*. Maximum number of isolates were from female patients (59.6%) compared to male (40.4%) patients [Table 1].

Table 1: Gender wise distribution of Enterococcus species

Gender Wise Distribution				
Gender	***E. Faecalis***	***E. Faecium***	***E. Avium***	**Total (%)**
Male	21	15	6	42 (40.4%)
Female	27	27	8	62 (59.6%)
Total	48	42	14	104 (100%)

Among these, 51% of the samples were received from In-patient department (IPD) and 49% were from Outpatient department (OPD). Majority of the isolates (33.7%) were identified in the age group of <10 years followed by 21-30 years (14.4%) [Table 2].

Table 2: Age wise distribution of study population

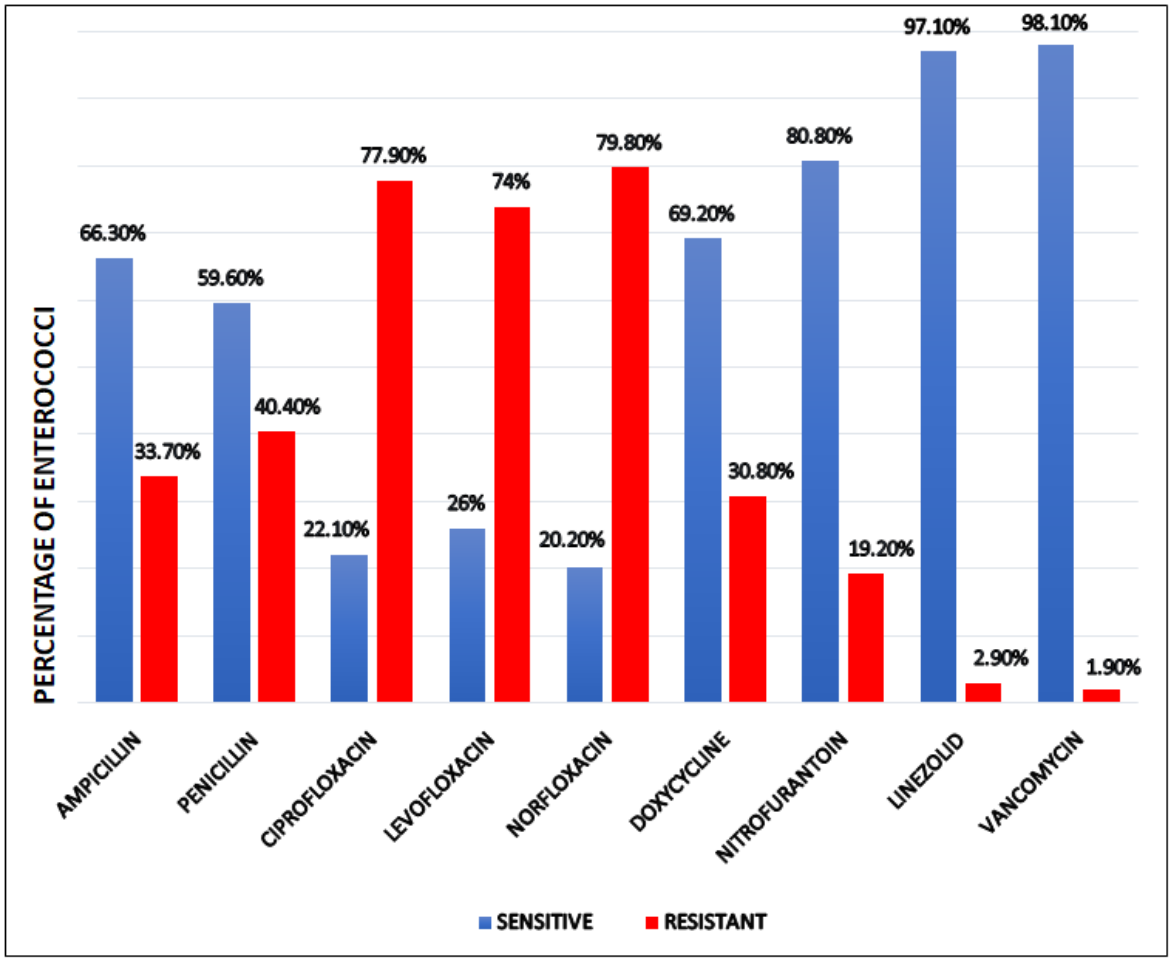
Age Wise Distribution			
Age In Years	**Male**	**Female**	**Total %**
< 10	22	13	35 (33.7%)
11-20	2	8	10 (9.6%)
21-30	2	13	15 (14.4%)
31-40	3	10	13 (12.5%)
41-50	3	7	10 (9.6%)
51-60	4	6	10 (9.6%)
>60	6	5	11 (10.6%)
TOTAL	42	62	104 (100%)

Out of these 104 isolates, 83 isolates (79.8%) were resistant to norfloxacin, 81 (77.9%) were resistant to ciprofloxacin, 77(74%) were resistant to levofloxacin and 42 (40.4%) were resistant to penicillin. 102 isolates (98.1%) were sensitive to vancomycin, 101 (97.1%) were sensitive to linezolid, followed by 84 (80.8%) to nitrofurantoin [Figure 1]. *E. faecium* strains as compared to *E. faecalis* and *E. avium* displayed a higher degree of drug resistance to the antibiotics [Table 3].

Table 3: Antimicrobial susceptibility pattern of different Enterococcus species

Antibiotics	***E. Faecalis* (N=48)**		***E. Faecium*(N=42)**		***E. Avium*(N=14)**	
	Sensitive	**Resistant**	**Sensitive**	**Resistant**	**Sensitive**	**Resistant**
Ampicillin	40 (83.3%)	8 (16.7%)	18 (42.9%)	24 (57.1%)	11 (78.6%)	03 (21.4%)
Penicillin	33 (68.8%)	15 (31.2%)	18 (42.9%)	24 (57.1%)	11 (78.6%)	03 (21.4%)
Ciprofloxacin	12 (25%)	36 (75%)	05 (11.9%)	37 (88.1%)	06 (42.9%)	08 (57.1%)
Levofloxacin	14 (29.2%)	34 (70.8%)	06 (14.3%)	36 (85.7%)	07 (50%)	07 (50%)
Norfloxacin	11 (22.9%)	37 (77.1%)	05 (11.9%)	37 (88.1%)	05 (35.7%)	09 (64.3%)
Doxycycline	30 (62.5%)	18 (37.5%)	31 (73.8%)	11 (26.2%)	11 (78.6%)	03 (21.4%)
Nitrofurantoin	40 (83.3%)	08 (16.7%)	30 (71.4%)	12 (28.6%)	14 (100%)	0 (0%)
Linezolid	46 (95.8%)	02 (4.2%)	41 (97.6%)	01 (2.4%)	14 (100%)	0 (0%)
Vancomycin	47 (97.9%)	1 (2.1%)	41 (97.6%)	01 (2.4%)	0 (0%)	0 (0%)

Figure 1: Antibiogram of Enterococcal isolates from urine



E. faecium strains were found more resistant to ampicillin (p=0.000), penicillin (p=0.013), ciprofloxacin (p=0.043) and levofloxacin (p=0.024), which was statistically significant. HLAR was detected in 53 (51%) of the isolates which showed high level resistance to gentamicin and/or streptomycin by high content disc diffusion. 43 (41.3%) were high-level gentamicin resistance (HLGR), 36 (34.6%) were high-level streptomycin resistance (HLSR) and 26 (25%) were both HLGR and HLSR. High level resistance to gentamicin and streptomycin among *E. faecalis* strains were 41.7% and 39.6%, respectively, and high-level resistance to gentamicin and streptomycin among *E. faecium* strains were 52.4% and 35.7%, respectively. Combined resistance to both the aminoglycosides was slightly higher in *E. faecalis* (29.2%) as compared with *E. faecium* (26.2%) [Table 4].

Table 4: Distribution of High-Level Aminoglycoside Resistance (HLAR) among Enterococci

HLAR	*E. faecalis* (n=48)	*E. faecium* (n=42)	*E. avium* (n=14)	TOTAL
HLGR	20 (41.7%)	22 (52.4%)	01 (7.1%)	43 (41.3%)
HLSR	19 (39.6%)	15 (35.7%)	02 (14.3%)	36 (34.6%)
HLGR + HLSR	14 (29.2%)	11 (26.2%)	01 (7.1%)	26 (25%)

*HLGR=High-Level Gentamycin Resistance, HLSR=High-Level Streptomycin Resistance

The frequency of HLAR (p = 0.008), especially HLGR (p = 0.012) was found more in *E. faecium* as compared to *E. faecalis*, which was statistically significant.

Among these 104 enterococcal isolates, 2 isolates (1.9%) were found to be VRE (MIC≥256µg/ml) and 102 (98.1%) were vancomycin sensitive enterococcus. Of the two isolates one was *E. faecalis* and the other was *E. faecium*. The VRE isolates were found to be sensitive to linezolid and high-level aminoglycosides. Multidrug resistance (defined as nonsusceptibility to at least one agent in three or more antimicrobial categories) was seen in 27 (25.9%) enterococcal isolatesand out of these, 15 (35.7%) were *E. faecium* strains. Among HLAR *E. faecium* and *E. faecalis* isolates, 13 (30.9%) and 7 (14.6%) multidrug-resistant isolates were recognized, respectively.

DISCUSSION

Recent years have witnessed increased interest in enterococci not only because of their ability to cause serious infections but also because of their increasing

resistance to many antimicrobial agents. The present study provides an estimate of the recent pattern of species distribution and antimicrobial susceptibility pattern of enterococcal isolates in urine in an Indian tertiary care hospital. The overall prevalence of Enterococcal infection varies across continents, countries and within hospitals. Incidence of UTI due to *Enterococcus* spp. in India varies from 0.5%-44.06% among different population. [14,15]In the present study, prevalence rate of Enterococci isolated from urine was 4.53%, which was consistent with the study done by Wavare SM et al., in Mumbai (4.2%) from UTI patients.[16]Das S, in Kolkata showed prevalence rate of 10%, whereas Kaur N et al., in Haryanareported an enterococcal isolation rate of 33% from urine samples.[17,18]Reasons for these higher urinary isolates than present study include active surveillance for enterococcal infection and the differentiation between colonization and infection might not be properly carried out.[3]The present study shows a high incidence of enterococcal UTI among females (59.6%) compared to males (40.4%), which could be due to close proximity of anal orifice and urethra in females or due to poor hygiene. Similar findings were also found in other studies. [6,19,20]Frequency of enterococci isolated was predominately from hospitalised patients (51%) than out-patients (49%), as has been observed in other studies from India. [18,19,21]In the present study, the maximum percentage of isolation was seen among the age group <10 years (33.7%) followed by 21-30 years (14.4%). This result was in accordance with the study of Karna A et al. [22]

In today's era, correct speciation is very important as the different Enterococcal species shows varied resistance to antibiotics. *E. faecalis* (46.1%) as the overall predominant isolate is congruent with previously published literature.[6,15,16,21,23]The proportion of *E. faecium* (40.4%), however, also appears to be high in our institute. This might be due to the increased use of antibiotics expected in a tertiary care institute such as ours which selects out the more resistant species. Recent studies from India and outside have also reported rising rates of *E.faecium* as high as 48.3% to 55.4%. [4,24]At other places, however, *E. faecium* still constitutes only approximately 4 to 10% of the enterococcal isolates. [6,25]*E. avium* was found as the predominant non-*E. faecalis* and non-*E. faecium* isolate in the current study comprising of 13.5% of the total isolates, which have also been reported from India. [4,14,16,23]A high resistance rate to various antimicrobials (norfloxacin, ciprofloxacin, levofloxacin and penicillin) was observed in the current study which is a cause of concern and precludes their use in routine treatment of enterococcal infections in this region. Similar high resistance to fluoroquinolones was also noted by other studies in India, Egypt and Iran. [6, 14,16.24]On the other hand, low resistance was observed to nitrofurantoin (19.2%), linezolid (2.8%) and vancomycin (1.9%). Our study has revealed very encouraging results for nitrofurantoin as 80.8% of our isolates were susceptible, which is in concordance with other studies from India. [16,17,23]These latter antimicrobials may therefore be indicated for treatment of enterococcal infections, especially nitrofurantoin may be recommended for empirical treatment of urinary tract infection due to *Enterococcus* species in our region. Although *E. faecalis* plays an important role in generating nosocomial infections because of its high binding potency and its proliferation in the intestine, *E. faecium* can more easily acquire antibiotic resistance. [3]In agreement with previous studies[14,20], we found that *E. faecium* strains displayed a higher degree of drug resistance to antibiotics in comparison to *E. faecalis.*

HLAR was observed in 51% isolates (HLGR in 41.3% and HLSR in 34.6%) in our study. Frequency of HLAR in India ranges from 46% to 88.6%. [26,27]HLAR was observed more in *E. faecium* isolates (62%) than other species. Similar findings were observed by studies in India, where HLAR among *E. faecium* isolates was found significantly higher than *E.faecalis*. [14,26]HLGR was detected in 41.7% in *E.faecalis* and 52.4% in *E. faecium*which was in accordance with the study by Rana D et al. and S. Sreeja et al. [28,29]HLSR was detected in 39.6% in *E.faecalis* and 35.7% in *E. faecium*which was in accordance with the study by Huidrom S et al. and Rana D et al.[27,28]There was a higher percentage positivity of the isolates having HLGR (41.3%) in comparison to the isolates having HLSR (34.6%). Previous studies in India have reported that HLGR was more common than HLSR in all species of isolated enterococci respectively. [28,30]HLAR is due to release of various aminoglycoside modifying enzymes. Combination therapy with cell wall active agents (penicillin, ampicillin or vancomycin) and an aminoglycoside is recommended for the treatment of serious enterococcal infections. However, HLAR will nullify the efficacy of this combination. Since enterococcal resistance to gentamicin and streptomycin occur by different mechanisms of enzymatic inactivation, it is important to test susceptibilities to both agents.

In our study, two isolates were found to be Vancomycin resistant with MIC ≥ 256µg/ml. VRE observed in the present study (1.9%) is similar to the study by Barman J et al. (1%), though lower than in the study by Phukan C et al. (24%) done in Assam. [21,23]VRE frequency in other studies outside India ranges from 4.5 to 21%. [6,24]Vancomycin resistance was not found to be a major resistance in this region, but VRE appears to be an emerging pathogen in India. In the present study, 25.9% of enterococci exhibited multidrug resistance (MDR).Abimannan GC et al. reported 17.95% MRD enterococci [19], whereas other studies in India reported a high prevalence of MDR enterococcal isolates ranging from 31.9% - 63%. [20,22]The proportion of MDR was found to be more

prevalent among *E. faecium* (35.7%), as compared to *E. faecalis* (20.8%) and HLAR isolates than non-HLAR isolates. This result is more likely comparable with the other studies, in which higher prevalence rate of MDR was observed among *E. faecium* and HLAR respectively. [20]However, the overall incidence of MDR in our centre was lower than that in other studies. [20,22]Irregular administration of antibiotics targeting sensitive strains promotes the emergence of resistant strains, especially MDR strains, with the ability to colonize the gut lumens of patients, which leads to an increase in the direct and indirect transfer of the genetic material of resistant strains. Recently, significant increases in the prevalence of resistant enterococci with the MDR phenotype have been reported in the USA, Europeand other developed countries.

LIMITATIONS OF THE STUDY

Due to resource constraints and cost, molecular characterization of the enterococcal strains was not done in this study.

CONCLUSION

This study highlights the emergence of multidrug-resistant enterococci, especially in high-level aminoglycoside which poses a serious therapeutic challenge among clinicians. The high detection rate of HLAR among the studied Enterococcus species and the coexistence of HLGR and HLSR strains provide crucial insights into the necessity of HLAR testing as a routine microbiology procedure. Although the frequency of isolation of VRE is not very high in our centre, monitoring of VRE is the need of hour since it appears to be an emerging pathogen in India. Proper implementation of an efficient infection control program and regular surveillance of antimicrobial resistance of enterococci is needed in order to establish a rational antibiotic policy for the better management of enterococcal urinary tract infections.

ACKNOWLEDGEMENTS

We would like to sincerely thank the technical staff of the department of Microbiology, Tezpur Medical College and Hospital, Assam, India for their support and co-operation.

SOURCE(S) OF SUPPORT

Nil

CONFLICTING INTEREST (IF PRESENT, GIVE MORE DETAILS)

Nil

ETHICAL POLICY AND INSTITUTIONAL REVIEW BOARD STATEMENT

This study was approved by Institutional Ethical Committee (IEC No:05/TMCH) of Tezpur Medical College and Hospital, Govt. of Assam, Ministry of Health and Family Welfare on 16th December 2016. The study adhered to the Declaration of Helsinki, 2013, and all participants provided consent before inclusion.

PATIENT CONSENT STATEMENT

Prior to commencing the study, written parental/guardian consent and/or patient consent was obtained for each study participant before data collection.

REFERENCES

1. Paul D, Anto N, Bhardwaj M, Prendiville A, Elangovan R, Bachmann TT, et al. Antimicrobial resistance in patients with suspected urinary tract infections in primary care in Assam, India. JAC Antimicrob Resist2021;3(4):1-7
2. Texeira LM, Facklam RR. Enterococcus. In: Borriello SP, Murray PR, Funke G, editors. Topley& Wilsons Microbiology & Microbial Infections. Bacteriology Vol. 2. 10th ed. United Kingdom: Wiley; 2005:882–902
3. AgudeloHiguita NI, Huycke MM. Enterococcal disease, epidemiology, and implications for treatment. In: Gilmore MS, Clewell DB, Ike Y, Shankar N, editors. Enterococci: From Commensals to Leading Causes of Drug Resistant Infection. Boston: Massachusetts Eye and Ear Infirmary; 2014. p. 1-27.
4. Padmasini E, Padmaraj R, Ramesh SS. High level aminoglycoside resistance and distribution of aminoglycoside resistant genes among clinical isolates of Enterococcus species in Chennai, India. Scientific World Journal 2014; 2014:329157
5. Hollenbeck BL, Rice LB. Intrinsic and acquired resistance mechanisms in enterococcus. Virulence2012;3(5):421-33
6. Haghi F, Lohrasbi V, Zeighami H. High incidence of virulence determinants, aminoglycoside and vancomycin resistance in enterococci isolated from hospitalized patients in Northwest Iran. BMC Infect Dis 2019;19(1):744
7. Cetinkaya Y, Falk P, Mayhall CG. Vancomycin-resistant enterococci. ClinMicrobiol Rev 2000;13(4):686-707.
8. Vidyalakshmi P, Gopalakrishnan R, Ramasubramanian V, Ghafur KA, Nambi PS, Thirunarayana M. Clinical, epidemiological, and microbiological profile of patients with vancomycin-resistant enterococci from a tertiary care hospital. J Glob Infect Dis 2012; 4(2):137-8.
9. Lucas GM, Lechtzin N, Puryear DW, Yau LL, Flexner CW, Moore RD. Vancomycin-resistant and vancomycin-susceptible enterococcalbacteremia: comparison of clinical features and outcomes. Clin Infect Dis 1998;26(5):1127-1133.
10. Wong AH, Wenzel RP, Edmond MB. Epidemiology of bacteriuria caused by vancomycin-resistant enterococci—a retrospective study. Am J Infect Control 2000; 28(4): 277–281
11. Facklam RR, Collins MD. Identification of Enterococcus species isolated from human infections by a conventional test scheme. J ClinMicrobiol 1989;27(4):731-4.
12. Collee JG, Miles RS, Wan B. Tests for the identification of bacteria. In: Collee JG, Fraser AG, Marmion BP, Simmons A, editors. Mackie and

McCartney practical medical microbiology. 14th ed. Edinburgh: Churchill Livingstone; 1996. p. 131–50
13. CLSI. Performance Standards for Antimicrobial Susceptibility Testing. 27th ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute; 2017.
14. Goel V, Kumar D, Kumar R, Mathur P, Singh S. Community acquired Enterococcal urinary tract infections and antibiotic resistance profile in North India. J Lab Physicians 2016;8(1):50-4
15. Das S, Konar J, Talukdar M. Prevalence of vancomycin-resistant Enterococcus causing urinary tract infection in a tertiary care hospital of Eastern India. Biomed Biotechnol Res J 2021;5:463-5
16. Wavare SM, Ghorpade MV, Gajul SV, Sajjan AG, Karigoudar RM. A study of Vancomycin resistant Enterococci isolated from urinary tract infections. Int J Pharm PharmSci 2015;7(5):337-9.
17. Das S, Konar J. Role of Enterococcus in nosocomial urinary tract infection in a tertiary care hospital in West Bengal. IOSR Journal of Dental and Medical Sciences (IOSR-JDMS) 2017;16(12):79-81.
18. Kaur N, Chaudhary U, Aggarwal R, Bala K. Emergence of VRE and their antimicrobial sensitivity pattern in a tertiary care teaching hospital. J Med BiolSci 2009; 8:26-32
19. Abimannan GC, Ramakrishna MS, Jeyamani L, Anbalagan K, Ramalingam A. Emergence of Drug Resistant Enterococcus Species from Patients with Urinary Tract Infections in a Tertiary Care Centre-A Retrospective Analysis. National Journal of Laboratory Medicine 2021 ;10(3):09-13
20. Bhatt P, Patel A, Sahni AK, Praharaj AK, Grover N, Chaudhari CN, et al. Emergence of multidrug resistant enterococci at a tertiary care centre. Med J Armed Forces India 2015;71(2):139-44
21. Phukan C, Lahkar M, Ranotkar S, Saikia KK. Emergence of van A gene among vancomycin resistant enterococci in a tertiary care hospital of North-East India. Indian J Med Res2016; 143:357-61
22. Karna A, Baral R, Khanal B. Characterization of clinical isolates of enterococci with special reference to glycopeptide susceptibility at a tertiary care center of Eastern Nepal. Int J Microbiol 2019(5):01-08
23. Barman J, Nath R, Saikia L. Drug resistance in Enterococcus species in a tertiary care hospital in Assam, India. Indian J Med Res2016; 143:107-110.
24. Gök ŞM, TürkDağı H, Kara F, Arslan U, Fındık D. Investigation of antibiotic resistance and virulence factors of Enterococcus faecium and Enterococcus faecalis strains isolated from clinical samples. MikrobiyolBul 2020;54(01):26–39.Turkish
25. Kateete DP, Edolu M, Kigozi E, Kisukye J, Baluku H, Mwiine FN, et al. Species, antibiotic susceptibility profiles and van gene frequencies among enterococci isolated from patients at Mulago National Referral Hospital in Kampala, Uganda. BMC infectious diseases. 2019;19(1):486.
26. 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; 26:369-71.
27. Huidrom S, Narayanswamy G, Dadlani R. Detection of highlevel aminoglycoside resistant pattern of Enterococci isolated from urine samples at a tertiary care hospital in Bengaluru. Ann Trop Med Public Health 2016; 9(3):165-9
28. Rana D, Sande S. Study of prevalence and antimicrobial susceptibility pattern of enterococci isolated from clinically relevant samples with special reference to high Level aminoglycoside resistance (HLAR) in a rural tertiary care hospital. J Evolution Med Dent Sci 2020;9(34):2472-2478
29. Sreeja S, Babu SPR, Prathab AG. The prevalence and the characterization of the Enterococcus species from various clinical samples in a tertiary care hospital. Journal of Clinical and Diagnostic Research 2012; 6(9):1486-8
30. Parameswarappa J, Basavaraj VP, Basavaraj CM. Isolation, identification, and antibiogram of Enterococci isolated from patients with urinary tract infection. Annals of African Medicine 2013;12(3):176-81