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Prevalence and Susceptibility Pattern of *Enterococcus* spp in Clinical Samples: A Retrospective Study From a Tertiary Care Teaching Hospital in an Eastern Indian State

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ABSTRACT

Background: Enterococcus, a low virulent yet hardy organism, is a cause of many community acquired as well as nosocomial infections. Antibiotic resistance in *Enterococcus* spp. is rising worldwide owing to their intrinsic resistance to multiple drugs. The combination therapy of beta-lactam antibiotics with aminoglycosides is the choice of treatment for this type of infection. But this is often rendered ineffective on account of high-level aminoglycoside resistance. Vancomycin resistance further complicates the scenario.
Objectives: To note the predominant infections caused by *Enterococcus* spp. and to show their resistance pattern, with particular emphasis on vancomycin resistance.
Materials and methods: This study was conducted retrospectively in our tertiary care teaching hospital in Odisha, Eastern India, where 200 consecutive, nonrepetitive *Enterococcus* spp obtained on culture were included. Their demographic profile was collected from the lab register, and analysis was done using MS Excel.
Results: The commonest sample from which *Enterococcus* spp was isolated was urine (n = 82, 41%), followed by blood (n = 49, 24.5%). *E. faecalis* (n = 120, 60 %) followed by *E. faecium* (n = 55, 27.5 %) were the most common species seen. Flouroquinolones, macrolides, and tetracycline were the most resistant antibiotics for all the *Enterococcus* species. *E. faecalis* had a much higher percentage of susceptibility to penicillin and higher level gentamicin (76.5% and 55.6%, respectively) compared to *E. faecium* (10.7% and 13.2%, respectively). Among the total, 43 (21.5%) isolates were vancomycin resistant, and only 3 (1.5%) showed moderate susceptibility. All the isolates 200 (100%) were tigecycline susceptible.
Conclusion: The present study highlights increased vancomycin resistance as noted in 21.5% Enterococcus isolates. Quinolones, macrolides, and tetracycline showed better sensitivity to vancomycin resistant Enterococcus, probably owing to lesser use in clinical scenarios. Urinary tract infection is the predominant infection caused by *Enterococcus* spp. Nitrofurantoin is an effective drug, particularly for *E. faecalis*
Keywords: *Enterococcus* spp.; Vancomycin resistant Enterococcus (VRE); *E. faecalis*; *E. faecium*

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INTRODUCTION

Enterococci belong to the family Enterococcaceae, which are facultatively anaerobic Gram positive cocci appearing singly, in pairs, or in short chains that are catalase negative, oxidase negative, organ-

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isms [1]. They survive in hostile conditions like 6.5% NaCl, 5 to 65°C temperature, pH-4.5 to 10, and hydrolyze of esculin even with 40% bile [1]. These are part of the bacterial flora of the intestine and are low-virulence organisms that produce disease when there is any breach in the gastrointestinal or genitourinary tracts mucosa and also in cases of immunosuppression. They have been known to cause sepsis, endocarditis, biliary tract infections, urinary tract infections, wound infections, and intra-abdominal abscesses [2]. *E. faecium* and *E. faecalis* cause more than 95% of these infections. At the same time, other species encountered uncommonly are- *E. casseliflavus, E. gallinarum, E. avium, E durans, E. hirae* etc [1, 3].

This organism belongs to ESKAPE (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumanii, Pseudomonas aeruginosa* and *Enterobacter* spp) pathogens, as it is the second predominant cause for nosocomial infections across the world [4]. Resistance to many antimicrobial agents helps the organism to survive with a selective advantage over other organisms in a hospital environment where antibiotics are rampantly used [5].

Antibiotic resistance to drugs like cotrimoxazole, aminoglycosides (except high level aminoglycosides), and cephalosporin among *Enterococcus* spp. are rising worldwide, thus posing a great challenge for their treatment [6]. Infections by these are generally treated with a combination therapy of cell wall active antibiotics and aminoglycosides. However, the emergence of high level aminoglycoside resistance has made treatment with this synergistic with a high-level combination even more challenging [6].

Many of the strains of *Enterococcus* spp. now exhibit resistance to glycopepetides (e.g. vancomycin). Vancomycin is the drug of choice for the organism with in vitro resistance to the choicest first line combination therapy. The prevalence of vancomycin resistant *Enterococci* (VRE) is increasing worldwide [7]. Studies from India show a widespread variation in the prevalence of VRE, ranging from 0-30% with an overall prevalence of 12.4%, and studies have shown a steady increase from 4.8% in 2010 to 14.1% in 2020 [8]. Enterococci are known reservoirs of antibiotic resistance genes and effectively transfer them to different bacteria, like methicillin resistant *Staphylococcus aureus* [9]. Owing to the controversy in the literature regarding the prevalence of vancomycin resistance and the lack of adequate data from our locality, noting the prevalence in the locality at hand is an essential task at hand. The present study was undertaken to determine the current status of infection caused by *Enterococcus* spp. and denote their resistance pattern in our tertiary care teaching hospital in Odisha, Eastern India.

MATERIALS AND METHODS

This study was conducted retrospectively over six months from October 2021 to March 2022 in our 1400 bed tertiary care hospital, catering to low - middle-income group patients in Bhubaneswar, Odisha. All the specialties and super specialties are in the hospital premises, and the central lab caters to all the clinical samples from all the areas. Clinical samples growing *Enterococcus* were included consecutively. Repeated samples from the same patient and the same site were excluded from the study. Data collection for relevant information like patient demographics, source of infection, etc was collected from the laboratory register.

All samples were collected by trained personnel maintaining proper aseptic precautions according to the standard operating procedures (SOP) and transported to the laboratory within 1 hour of collection in designated sample collection boxes in cold chain. In case of delay, the samples were stored in the refrigerator, except CSF which was stored at 37°C and was sent to the laboratory within 4 hours. All the samples were put to culture and staining procedures immediately upon receipt in the laboratory. Blood culture was done in Bac T Alert, Biomerieux. All other specimens were cultured on blood and Macconkey agar media, and urine was cultured on CLED agar media. Growth obtained following overnight incubation at 37°C in ambient air was identified primarily as *Enterococcus* spp. according to the colony morphology, Gram staining, and biochemical properties. On a blood agar plate, *Enterococcus* produced round, small, transparent, smooth, colonies and on the MacConkey agar medium, they produced tiny pink colonies. These colonies subsequently were Gram positive, catalase negative, and bile esculin positive. Further species identification and susceptibility were done in Vitek-2, Biomerieux. The MIC of vancomycin obtained from Vitek 2 was interpreted as per their MIC for the drug as sensitive ≤ 4 μg/ml, intermediate- 8-16 μg/ml, and resistant- ≥ 32 μg/ml [10]. The patient demographics, and antibiotic susceptibility pattern of the isolates were compared between cases (vancomycin MIC ≥ 8 μg/ml) and controls (vancomycin MIC < 8 μg/ml).

The sample size was determined using a single population proportion formula considering the 10% prevalence of vancomycin resistant Enterococcus in clinical samples, a marginal error of 5%, and a 95% confidence interval of 1.96 using the following sample size determination formula. The samples were collected using systematic random sampling. The number of samples required was 138, but 200 samples were collected for ease of calculation.

Being a National Accreditation Board for Testing and Calibration Laboratories (NABL) accreditated lab, all standard operating procedures and quality control procedures were strictly followed. Sterility checking of all media was done before inoculation. ATCC *E. faecalis* 29212 was used as a control strain weekly for both identification and MIC performance of the Vitek 2 instrument and cards. The result of the Vitek 2 MIC was compared with the known MIC of the control strain [10]. The manufacturer's instructions were thoroughly followed for all maintenance requirements of the instrument. Data entry was done with MS Excel software. Bivariate and multivariate regression analysis was performed by Graph Pad Prism software. A probability value (P) of < 0.05 was considered statistically significant.

Ethics Approval and Consent to Participate

The study was approved by the Institutes ethics committee viano. IEC/IMS.SH/SOA/2O21/269. All the processes were within patient care standards. Written informed consent was obtained from all the patients or the immediate caregiver (whichever is applicable) during admission for all the procedures and sample collection as necessitated for therapeutic purposes. No patient data was disclosed during the study, and diagnostic or therapeutic activity was not hampered.

RESULTS

In this study, 119 (59.5%) samples were from males. The age of the patients ranged from 0 to 95 years, with a median age of 50 years. The mean and standard deviation of age

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are 48.8 ± 20.9 years. The predominant age group of these patients belonged to were 61-70 years old. Of the total samples, 86/200 (43%) samples were from various inpatient departments, while 78/200 (39%) were from intensive care units (ICUs), and 36 (18%) were from outpatient departments (Table 1).

The most common sample from which *Enterococcus* spp. was isolated was urine (82, 41%), followed by blood (49, 24.5%). The most common species of *Enterococcus* isolated from the present study was *E. faecalis*, consisting of 60% of cases, followed by *E. faecium* in 27.5% of samples. The other species isolated were *E. gallinarum* (8%), *E. casseliflavus* (2.5%), and *E.avium* (2%), as shown in Table 2.

E. faecium had a high percentage of resistance to penicillin (89.3%), while all other species had higher susceptibility to penicillin and were not β-lactamase producing. Flouroquinolones, macrolides, and tetracycline were the most resistant antibiotics for all the *Enterococcus* species. High level gentamycin resistance was detected in 80% of *E. casseliflavus*, 76.8% of *E. faecium*, and 50% of *E. gallinarum*, while it was much lower (45.4%) in *E. faecalis* and not detected in *E.avium* isolates. Vancomycin resistance was seen in 21.5% of isolates of *Enterococcus* spp., and 1.5% were vancomycin intermediates. *E. faecalis* and *E. faecium* are resistant to vancomycin in 17.6% and 21.4% of cases, respectively. *E. gallinarum* and *E. casseliflavus*, intrinsically resistant to vancomycin, were reported as VRE in 100% of isolates. Similarly, linezolid was sensitive in 89.9% of *E. faecalis* isolates, marginally lower than *E. faecium* (92.9%) isolates. Teicoplanin was a sensitive antibiotic in most strains of Enterococcus. The isolates of *E. casseliflavus* had a higher percentage of resistance to linezolid (80%) and teicoplanin (60%) than other species. *E. faecalis* daptomycin had a high proportion of intermediate (47.9%) isolates. The most sensitive antibiotic for treating *Enterococcus* spp. was tigecycline, which showed 100% sensitivity for all the isolates (Figure 1).

The demographics of the patients from whom VRE and vancomycin sensitive Enterococcus (VSE) were obtained were compared. As per multivariate regression analysis, the age groups of 21-30 and 51-60 years have a higher chance (OR > 1) of isolating VRE than vancomycin sensitive Enterococcus (VSE). Although males were affected more commonly than females in our study, there was no significant difference or association of VRE with gender. ICU admission is also associated with higher odds of isolation of VRE (OR- 1.22) as shown in Table 1. Among various samples, bile (50%) and wound swabs (38.1%) were the common samples from which VRE was isolated (Table 2).

Among the vancomycin intermediate isolates (MIC = 8; n = 3), 33.3% were linezolid resistant, while all the other antibiotics were susceptible in these three and none showed high levels of gentamicin resistance. Among the vancomycin resistant isolates, 48.8% were resistant to linezolid. Teicoplanin and daptomycin were resistant to 37.2% and 34.9% of VRE isolates, respectively. Other tested antibiotics showed much less resistance in the case of VRE. Only 18.6% of the VRE isolates also showed a high level of gentamicin resistance. There was a significant difference in the resistance shown by VRE and VSE isolates to different antibiotics (P- value < 0.01) except nitrofurantoin. Levofloxacin, ciprofloxacin, erythromycin, and tetracycline have a higher susceptibility chance in VRE isolates than in VSE. However, second line drugs like linezolid and teicoplanin have lower *in vitro* susceptibility in VRE cases (Table 3).

DISCUSSION

In this study, *Enterococcus* spp. was commonly from urine specimens, followed by blood and wound swabs. This is similar to the findings of studies from Ethiopia and India [11, 12]. This indicated that *Enterococci* mainly cause bacteremia, urinary tract infections (UTI), and wound infections. This organism is a normal flora of the intestine; thus, the proximity of the anal opening to the urethra, particularly in females, is the cause of the high chance of UTI by this organism.

In the present study, 82% of the samples were from various admitted patients (inpatients and ICUs). Manimala *et al.* [13] have also reported 69% isolation of *Enterococcus* from admitted patients. This may be because hospitalized patients are usually immunologically weak and prone to acquiring infections in a hospital environment.

The predominant age group to which these patients belonged in our study was 61-70 years old. In other studies [14], the maximum percentage of isolates was from patients between the age groups of 41 and 60 years. In the present study, males outnumbered females, with a male: females being 115: 85. This does not agree with most other studies [14] where females were the predominant sex. Community acquired UTI is the most common infection caused by *Enterococcus* occurs mostly in females and leads to a preponderance of female sex in most studies. However, in our study, most samples were from inpatients and ICUs. Thus we probably had hospital acquired infection caused by this organism. The study's retrospective nature limited us from having more clinical data to explain with certainty the origin of the infection (community or hospital acquired).

The most common species isolated in this study was *E. faecalis* (n = 120, 60%) followed by *E. faecium* (n = 55, 27.5%) while other species account for only 12.5% of strains isolated. This is in agreement with many previous studies [8, 15]. *E. faecalis* is the most common species of this genus (80-90%), causing clinical infections, while *E. faecium* accounts for a very low percentage (5-15%) [16]. However, there is a rise in *E. faecium* infections exemplified by a recent study where *E. faecium* presented as a major pathogen (74%) followed by *E. faecalis* (20%) in bloodstream infections with an overall mortality rate of 24% [17, 18].

Multidrug-resistant *Enterococci* are increasing as a silent epidemic. *Enterococci* show intrinsic resistance to beta lactam antibiotics and aminoglycosides. At the same time, they have acquired resistance from transposons and plasmids for glycopeptides, streptogramins, quinolones, tetracyclines, and macrolides [19, 20]. Penicillin, along with aminoglycosides is the treatment of choice for Enterococccal infections. In our study, resistance to benzylpenicillin was detected in 45.4% and 89.3% of *E. faecalis* and *E. faecium* isolates, respectively. A previous study from Eastern India [15] also reported that about 33% of isolates were resistant to ampicillin. On the other hand, most other studies from the Indian Peninsula report a very high level of ampicillin resistance, like 75% by Yadav *et al.* [12] and 70% by Mukherjee *et al.* [21]. Probably, the use of alternate drugs for this organism has led to this change in the scenario in our study.

Resistance to high level gentamicin means that combination therapy of beta lactams and aminoglycoside will no more be adequate for serious Enterococcal infections. High level aminoglycoside resistance (HLAR) in Enterococci is mostly due to the production of plasmid mediated aminoglycoside modifying enzymes and ribosomal mutations of antibiotic tar-

Table 1. Demographic information of the 200 participants.*

Characteristics	Total Number(%)	VRE Number(%)	VSE Number(%)	Bivariate analysis		Multivariate analysis	
				Correlation coefficient(95%CI)	P-value	Adjusted odds ratio(95%CI)	P-value
Age group							
0-10	8 (4)	0 (0)	8 (100)	1	NA	NA	NA
11-20	7 (3.5)	2 (28.6)	5 (71.4)	0.29 (0.14-0.98)	0.23	0.23 (0.11-0.67)	0.55
21-30	31 (15.5)	6 (19.4)	25 (80.6)	0.55 (0.23-1.64)	0.14	1.67 (0.2212.56)	0.25
31-40	25 (12.5)	4 (16)	21 (84)	0.48 (0.22-1.36)	0.06	0.32 (0.15-0.58)	0.12
41-50	29 (14.5)	8 (27.6)	21 (72.4)	2.36 (0.96-2.98)	0.02	3.24 (1.36-4.39)	0.13
51-60	35 (17.5)	11 (31.4)	24 (68.6)	1.33 (0.36-1.87)	0.36	1.36 (0.96-1.66)	0.11
61-70	38 (19)	9 (25.7)	26 (74.3)	0.69 (0.11-0.36)	0.15	0.55 (0.36-0.96)	0.72
> 70	27 (13.5)	3 (11.1)	24 (88.9)	0.11 (0.03-0.36)	0.36	0.33 (0.343.08)	0.22
Gender							
Male	119 (59.5)	28 (23.7)	90 (76.2)	1	0.63	0.59 (0.31-1.11)	0.07
Female	81(40.5)	15 (18.9)	64 (81.1)	0.727 (0.361-1.416)			
Patient Location							
ICU admission	78(39)	16 (20.8)	61 (79.2)	1.65 (0.98-2.36)	0.01	1.22 (0.88-1.65)	0.09
In patient	86(43)	20 (23.5)	65 (76.5)	0.56 (0.36-0.99)	0.25	0.65 (0.14-0.96)	0.21
Outpatient	36(18)	07(20.0)	28 (80.0)	1	NA	NA	

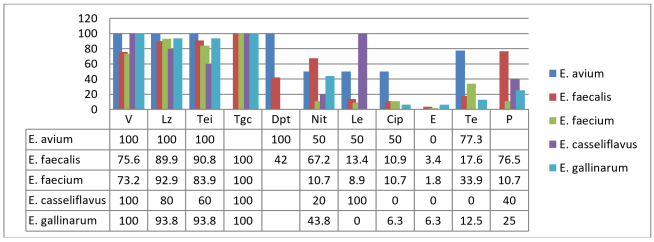
* VRE= Vancomycin resistant Enterococcus; VSE = Vancomycin sensitive Enterococcus; ICU = intensive care unit.

Table 2. Distribution of *Enterococcus* spp. according to the site of infection.*

Sample	No. of samples (% total)	*E.faecalis* No (% total in that sample)	*E.faecium* No (% total in that sample)	*E.gallinarum* No (% total in that sample)	*E.avium* No (% total in that sample)	*E.casseliflavus* No (% total in that sample)	*VRE* No (% total in that sample)
Bile	02 (1)	1 (50)	1 (50)	-	-	-	1 (50)
Blood	49 (24.5)	23 (46.9)	23 (46.9)	3 (6.1)	-	-	12 (24.4)
CSF	01 (0.5)	1 (100)	-	-	-	-	0 (0)
Respiratory samples (sputum and tracheal aspirates)	05 (2.5)	3 (60)	2 (40)	-	-	-	1 (20)
High vaginal swab	08 (4)	8 (100)	-	-	-	0 (0)	
Placental membrane	01 (0.5)	1 (100)	-	-	-	-	0 (0)
Pleural fluid	01 (0.5)	1 (100)	-	-	-	-	0 (0)
Pus	30 (15)	21 (70)	3 (10)	3 (10)	2 (6.7)	1 (3.3)	7 (23.3)
Urine	82 (41)	47 (57.3)	20 (24.4)	9 (10.9)	2 (2.4)	4 (4.9)	14 (17.1)
Wound swab	21 (10.5)	14 (66.7)	6 (28.6)	1 (4.8)	-	-	8 (38.1)
Total (% of total)	200 (100)	120 (60)	55 (27.5)	16 (8)	4 (2)	5 (2.5)	43 (21.5)

* VRE = vancomycin resistant Entrococcus and CSF = cerebrospinal fluid.

gets [22]. In our study, HLAR was noted in 45.4% and 76.8% of *E. faecalis* and *E. faecium* isolates, respectively. Other studies have noted similar findings [14, 15]. This calls for a profound hospital infection control awareness initiative for implementing hand hygiene, designated personal protective equipment in patient care areas as per anticipated procedure, frequent cleaning of rooms and bathrooms, and using private rooms for VRE patients to prevent dissemination. Vancomycin use should be justified and monitored by the clinician [23].



	V	Lz	Tei	Tgc	Dpt	Nit	Le	Cip	E	Te	P
E. avium	100	100	100		100	50	50	50	0	77.3	
E. faecalis	75.6	89.9	90.8	100	42	67.2	13.4	10.9	3.4	17.6	76.5
E. faecium	73.2	92.9	83.9	100		10.7	8.9	10.7	1.8	33.9	10.7
E. casseliflavus	100	80	60	100		20	100	0	0	0	40
E. gallinarum	100	93.8	93.8	100		43.8	0	6.3	6.3	12.5	25

Figure 1. Study flowchart. IVF = in vitro fertilization.

This study shows the high resistance in all isolates to tetracycline, quinolones, and macrolides, as in other studies [14, 21]. *Enterococcus* spp., in the present study, showed good susceptibility to linezolid and teicoplanin (about 90%). Previous studies from India and Bangladesh found no linezolid resistant *Enterococcus* isolates, denoting a rise in resistance to these drugs [24, 25]. Tigecycline was the most sensitive

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Table 3. Comparison of resistance pattern to different antibiotics among VRE, VSE and VISA isolates.*

MIC of vancomycin	VSE(<8) n =154 Total (%)	VIE (= 8) n = 3 Total (%)	VRE (≥ 8) n = 43 Total (%)	P- value
Linezolid	2 (1.3)	1 (33.3)	21 (48.8)	< 0.001
Teicoplanin	0 (0)	0 (0)	16 (37.2)	NA
Daptomycin	68 (44.2)	0 (0)	15 (34.9)	< 0.001
Nitrofurantoin	33 (21.4)	0 (0)	12 (27.9)	0.366
Levofloxacin	135 (87.7)	0 (0)	10 (23.3)	< 0.001
Ciprofloxacin	136 (88.3)	0 (0)	10 (23.3)	< 0.001
Erythromycin	147 (95.5)	0 (0)	10 (23.3)	< 0.001
Tetracycline	121 (78.6)	0 (0)	10 (23.3)	< 0.001
High Level Gentamicin	79 (51.3)	0 (0)	8 (18.6)	< 0.001
Benzyl penicillin	59 (38.3)	1 (33.3)	29 (67.4)	< 0.001

* VRE= Vancomycin resistant Enterococcus; VIE= Vancomycin intermediate Enterococcus; VSE= Vancomycin sensitive Enterococcus.

drug for *Enterococcus* spp. in the present study.

Vancomycin resistant *E. faecium* and *E. faecalis* strains were first detected in Europe in the 1980s and have been spreading globally since across different countries [26]. In the present study, 21.5% of isolates were vancomycin resistant. This is similar to other recent Indian studies [8, 14, 21]. A previous study in our locality estimated vancomycin resistance at 6.3% [15]. The mechanism of vancomycin resistance is alterated by altering the binding site of the drug D-Ala-D-Ala of cell wall peptidoglycan to D-Ala-D-Lac or D-Ala-D-Ser, thus reducing the binding affinity. This change is mediated commonly by chromosomal encoded or less often by plasmid mediated genetic elements like van A, B, C, D, E, F, G, L, M, and N [26]. van A pattern causes resistance to both vancomycin (MICs > 64 μg/mL) and teicoplanin (MICs > 16 μg/mL), while the van B pattern causes inducible resistance to vancomycin (MICs 32 to 64 μg/mL) but remains susceptible to teicoplanin [26]. the Van A is the most common genotype in India and worldwide [15]. In the present study, only 37.2% of VRE had concurrent resistance to teicoplanin, but no genetic study was conducted, which is a limitation of our work. Linezolid resistance was noted in 48.8% of VRE isolates in our work. Rising resistance to linezolid has also been noted in other studies from India [14]. This raises concern about limiting these reserve drugs' use in routine clinical practice. Due to the presence of both genes on the same conjugative plasmid, the erm B gene, which encodes macrolide resistance, co-transfers with the vancomycin resistance gene (van A)[26]. Our study noted about 24% concurrent resistance to macrolides among VRE. A 17% of urinary *Enterococcus* isolates were VRE. Recent Indian studies by Thakan *et al.* [14] and Atray *et al.* [27] also found that nitrofurantoin is useful for *Enterococcus* spp in urinary isolates. This drug was susceptible to 67.2% of *E. faecalis* isolates, while the sensitivity dropped markedly to about *E. faecium* (10.7%). Nitrofurantoin is resistant in about 28% of VRE isolates, too. Thus, the decision to use this drug in UTI needs to be carefully considered as per culture results.

CONCLUSION

The present study highlights increased resistance in *Enterococcus* spp. to all the antibiotics. We had a high prevalence of VRE isolates of 21.5%. There was a high prevalence of resistance to reserve drugs like linezolid, teicoplanin, and daptomycin in VRE isolates. At the same time, quinolones, macrolides, and tetracycline showed better sensitivity, probably owing to their lesser use in clinical scenarios. UTI is the predominant infection caused by *Enterococcus* spp., for which nitrofurantoin is effective in *E. faecalis* cases. Thus, infection prevention activity focusing on *Enterococcus* spp. is needed to mitigate the rising prevalence of VRE in hospital setting.

ETHICAL DECLARATIONS

Acknoweldgements

We acknowledge the support of IMS and SUM Hospital, SOA University for providing us with support to do the current work.

Ethics Approval and Consent to Participate

The study had was approved by the Institutes ethics committee via- no. IEC/IMS.SH/SOA/2O21/269. All the processes done were within patient care standards. Written informed consent was obtained from all the patients or the immediate caregiver (whichever is applicable) during admission for all the procedures and sample collection as necessitated for therapeutic purposes. No patient data was disclosed during the study, and diagnostic or therapeutic activity was not hampered.

Consent for Publication

Not applicable (no individual personal data included).

Availability of Data and Material

Data generated during this study are available from the corresponding author upon reasonable request.

Competing Interests

The authors declare that there is no conflict of interest.

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Authors' Contributions

Rout D and Bhattacharya S are responsible for the concepts, data acquisition, manuscript preparation, manuscript editing, and manuscript review. Bhoi P is responsible for

the concepts, design, data acquisition, and manuscript preparation. Sahu KK is responsible for the literature search, manuscript editing, and manuscript review. Panda NR is responsible for the design, manuscript preparation, and manuscript editing. Otta S is responsible for the design, definition of intellectual contents, literature search, manuscript preparation, manuscript editing, and manuscript review. All authors read and approved the final version of the manuscript.

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