Original Research

Detection of Vancomycin-Resistant Enterococcus in Clinical Settings in a Tertiary Care Hospital

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Abstract

Background: *Enterococcus* is a nosocomial infection that has become more prevalent due to its innate resistance to conventional antibiotics and recent acquisition of resistance to other current therapeutic alternatives. **Aim-** Detection of vancomycin-resistant *Enterococcus* in clinical settings in a tertiary care hospital.

Objective: The main objective of this study is to isolate and identify the antimicrobial susceptibility test pattern of the *Enterococci* from different samples and detect Vancomycin-resistant *Enterococcus* among the isolated *Enterococci* strains.

Material & Method: This study was conducted in the Bacteriological Section, Microbiology department, Teerthanker Mahaveer Medical College, Moradabad, from November 2023 to July 2024 .*Enterococcus* isolates from clinical samples received in the department of microbiology were included in the study. Isolates were identified and standard methods were used to determine the species. The antibiotic susceptibility test was done using Kirby Bauer's disc diffusion method. Vitek compact system was used to assessvancomycin's resistant & sensitive pattern.

Result: 135 isolates were identified as *Enterococcus* from 1677 clinicals amples like pus, urine, blood, body fluid, and foley's tip where 25 (18.5%) were resistant to vancomycin. The maximum age was 61-80 years and the minimum age was 18-20, from whom the isolates were discovered, among which females were 49 and males were 86. *Enterococcus faecalis* shows 16.2% resistance to the drug Vancomycin whereas *Enterococcus faecium* shows 24.5% resistance to the drug, whereas other species of *Enterococcus* found 100% sensitivity to the drug Vancomycin.

Conclusion: We conclude that infection control practices and active surveillance must be implemented. According to the prevalence data ofVancomycin-resistant *Enterococcus*, infection control measures must be strictly followed to prevent the spread of these infections. To reduce the Vancomycin-resistant *Enterococcus* rate, clinicians should be aware of this rising trend and put strict antibiotic policies and infection control protocols in place.

Keywords: Vancomycin-resistant *Enterococcus*, VITEK compact system, *Enterococcus faecalis*, *Enterococcus faecium*.

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Introduction

Enterococci are an indigenous normal flora of the GI system in both humans and animals. It is considered a facultative anaerobic gram-positive coccus. Numerous illnesses, including bacteremia, urinary tract infections,and endocarditis, thus both acquired in the community and hospitals are linked to it.[1] It is well known that Vancomycin-Resistant *Enterococcus* (VRE) is the most dangerous threat to public health and is considered one of the main sources of nosocomial infections.[2] The bactericidal action of the glycopeptide antibiotic vancomycin is based on its inhibition of peptidoglycan polymerisation in the bacterial cell wall. In the bacterial cell wall, long polymers of N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM) form a stiff peptidoglycan layer with a strongly cross-linked structure. Inhibiting glucosyltransferase (peptidoglycan synthase) and the P-phospholipid carrier, vancomycin binds to D-alanyl D-alanine, inhibiting the formation and polymerisation of NAM and NAG inside the peptidoglycan layer.[3] Bacterial cells die as a result of this inhibition, which weakens their cell walls and eventually allows internal components to flow out. The only bacteria that vancomycin can kill are gram-positive ones. There are two main ways that vancomycin-resistant enterococci (VRE) have become resistant:

1. Modified Target Sites: Vancomycin's binding affinity and, consequently, its efficacy are decreased when VRE substitutes D-alanyl-D-lactate or D-alanyl-D-serine for the D-alanyl-D-alanine terminal.
2. Gene Acquisition: Enterococci can create modified cell wall precursors by acquiring resistance genes, such vanA and vanB, from other bacteria, frequently via plasmids.[3]

VRE is a key source of rising issues in healthcare institutions, which has spread worldwide rapidly.[2] Thailand's National Antimicrobial Resistance Surveillance Centre reports that while the incidence of E. faecalis has remained constant for ten years (0.4% in 2018), that of E. faecium grew from 0.8% to 9.9% between 2012 and 2019.[4] In some of the study it was found that in 377 Enterococcus species, Enterococcus faecalis accounted for 239 (64.42%) of these isolates, while Enterococcus faecium made up 114 (30.72%). Other isolates included *Enterococcus durans* and *Enterococcus avium*. 24 (6.47%) of them were VRE.[5]

Depending on the region and kind of hospital, *Enterococcus faecium* (*E.faecium*) makes up most of VRE infections in nosocomial settings while *Enterococcus faecalis* (*E. faecalis*) makes up only 2–20% of VRE isolates.[6,7] Critically sick individuals hospitalized in the Intensive Care Unit (ICU) with a minor case had the greatest prevalence of VRE infection.[8] However, they can cause serious illnesses including meningitis and endocarditis. Rather than infecting organisms, the maximum number of clinical isolates of *Enterococci* is colonized.[9]

For most clinical isolates, including *Enterococci*, fast and accurate identification and susceptibility test results are to be obtained using an automated technique known as the VITEK 2. The main factor that serves as the basis for identification is biochemical processes. Also, the VITEK 2 system tracks an algorithm based on the growth kinetics that is used to determine MIC (Minimum Inhibitory Concentration) values.[10]

Material and Method

A cross-sectional study was conducted in the Teerthanker Mahaveer Medical College& Research Centre (TMMC&RC) Moradabad, from November 2023 to July 2024. A total of 1677 samples were taken for the study. Out of these, 135 samples were isolated and found to be *Enterococcus*, further species are identified along with their antimicrobial sensitivity testing through the Vitek compact system. Samples like blood, pus, urine, etc. were first collected. When a primary smear of the sample was made and stained with Gram stain, it was found to be gram-positive cocci which were oval-shaped and mainly observed in pairs. After that samples were processed on Blood agar, MacConkey agar & CLED agar. After a 24–48 hours incubation period, growth was observed and *Enterococcus* species were identified from the samples based on gram staining (gram-positive cocci), biochemical test (catalase negative), and bile esculin test (positive). Species identification was done. Antibiotic susceptibility testing by Kirby bauer disc diffusion method and VRE was detected by the Vitek compact system.[11]

VITEK 2 system: This integrated modular system called VITEK 2 (biome 'Rieux) includes a data terminal, reader-incubator apparatus, administrator module, filling-sealer unit, and multi-copy printer. This machine uses a fluorescence-based method to monitor changes in metabolism and bacterial growth inside the thin plastic micro-well cards. Biochemical substrates or antibiotics are present in different microwell cards. We used the VITEK 2 system's AST-P516 and ID-GPC cards to assess the enterococci antibiotic susceptibility. The ID-GPC card is a 64-well plastic card with 18 empty wells and 46 wells marked for fluorescence biochemical and inhibitory testing. The 20 antimicrobial compounds are present in the 64-well plastic AST-P5 16 card at varying concentrations.[12]

ANTIMICROBIAL	CONCENTRATION
Ampicillin-sulbactam	64 µg/ml
Benzyl-penicillin	64 µg/ml
Cefuroxime	8 µg/ml
Ciprofloxacin	4 µg/ml
Clindamycin	2 µg/ml
Erythromycin	2 µg/ml
Gentamicin, high level	150 µg/ml
Ampicillin	32 µg/ml
Imepenem	32 µg/ml
Levofloxacin	8 µg/ml
Nitro-furantoin	64 µg/ml
Norflaxacin	4 µg/ml
Oflaxacin	4 µg/ml
High level kanamycin	200 µg/ml
Teicoplanin	16 µg/ml
High-level streptomycin	200 µg/ml
Tetracycline	2 µg/ml
Trimethoprim-sulfamethoxazole	640 µg/ml
Vancomycin	6 µg/ml

In this investigation, we assessed the VITEK 2 system's performance in testing for High-level gentamicin resistance (HLGR) and High-level streptomycin resistance (HLSR) as well as ampicillin, vancomycin, and teicoplanin susceptibility alone.[9] According to the Clinical and Laboratory Standards Institute (CLSI) criteria, the antimicrobial sensitivity of the isolate was classified assensitive (S) and resistant (R).[10]

Results

This analysis was conducted in the Department of Microbiology at Teerthanker Mahaveer Medical College and Research Center, Moradabad. A total of 1677 samples were included in the project of which only 135 were found positive for the *Enterococcus* species within the time duration for the research project. So, the prevalence of the *Enterococcus* species in this hospital's patient sample was found to be 8.05%.Positive 135 (8.05%) Negative 1542 (91.95%) Total 1677 (100%)

Table 1: Showing prevalence of total isolated Enterococcus species.

Positive	135 (8.05%)
Negative	1542 (91.95%)
Total	1677 (100%)

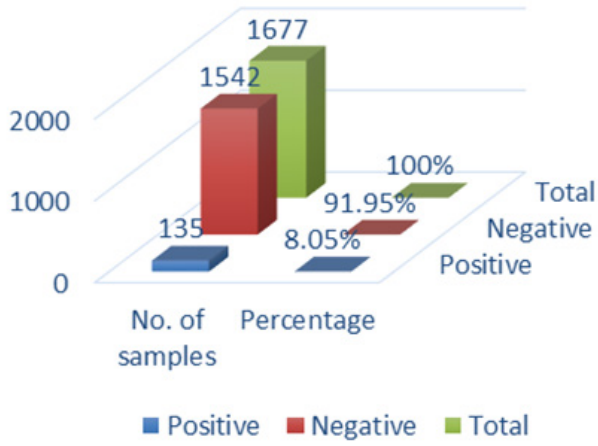

Figure 1: Showing the prevalence of total isolated Enterococcus species.

There were 135 samples found positive out of 1677 total samples, in which out of 135 positive samples for Enterococcus species, 74 (54.8%) were *E. faecalis*, 49 (36.3%) were *E. faecium*, and 12(8.9%) were other *Enterococcus* species. This is shown below in the table form.

Table 2: Showing data for isolated different Enterococcus

Species	Number of samples
Enterococcusfaecalis	74(54.8%)
Enterococcusfaecium	49(36.3%)
Other*Enterococcus*species	12(8.9%)

Figure 2: Showing data for isolated different Enterococcus species

A maximum number of samples found positive were from urine sample 62(45.9%) followed by pus 25(18.5%), blood 24(17.8%), body fluid, and foley's tip was in an equal number of 12(8.9%) as shown in the table below.

Table 3: Showing data for different types of samples

Specimen	Number
Urine	62(45.9%)
Pus	25 (18.5%)
Body Fluids	12(8.9%)
Blood	24 (17.8%)
Foley's Tip	12 (8.9%)

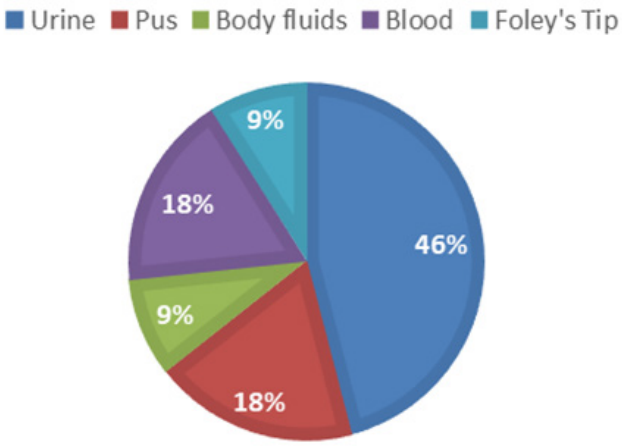

Figure 3: Showing data for different types of samples

In the present study, a total of 1677 samples were conducted in which only 135 samples were found positive for Enterococcus species. Out of 135 positive samples, there were 86 male patients and the rest 49 were female as shown in the table below.

Table 4: Gender wise distribution of samples

Gender	Number of samples
Male	86 (63.7%)
Female	49 (36.3%)
Total	135 (100%)

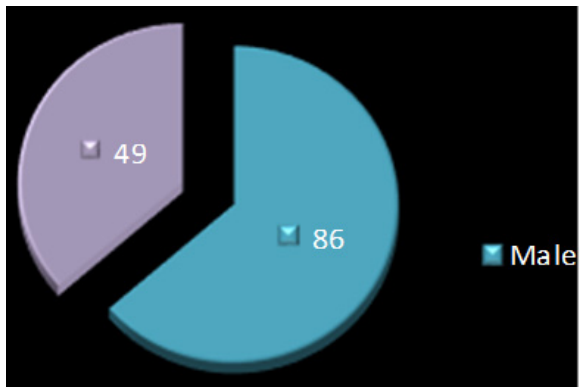

Figure 4: Gender-wise distribution of samples

This study was conducted on patients admitted to different wards of the hospital, which was divided into five groups according to their age group. Maximum number of *Enterococcus* species isolates were from 21-40 years age group. That was 62(45.9%) followed by 41-60 (36.3%), 61-80 and 18-20(8.9%), and 81-100 0% as shown in table below.

Table 5: Showing data for age group-wise distribution of samples

Age group	Total number	Percentage
18-20	12	8.9%
21-40	62	45.9%
41-60	49	36.3%
61-80	12	8.9%
81-100	0	0%

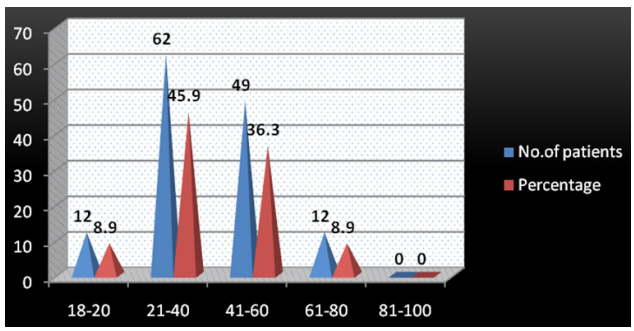

Figure 5: Showing data for age group-wise distribution of samples

Antimicrobial susceptibilities of 135 isolated *Enterococcus* species from various clinical samples as identified by VITEK2 Compact–fullyautomated and rapid ID/AST system. The MIC value was based on the reference microbrothdilution method as per CLSI and EUCAST guidelines-2024.

E. faecalis shows 16.2% resistance to the drug Vancomycin and *E.faecium* shows 24.5% resistance to the drug, whereas other species of *Enterococcus* found 100% sensitivity to the drug Vancomycin.

Table 6: AST data according to isolated Enterococcus species or different drugs

Species	Number of Enterococcus species	MIC breakpoints & interpretive Categories			Interpretation	
		S	I	R	S	R
E.*faecalis*	74(54.8%)	≤2	4	≥8	62(83.8%)	12(16.2%)
E.*faecium*	49(36.3%)	≤0.5	4	≥16	37(75.5%)	12 (24.5%)
Other *Enterococcus* species	12(8.9%)	≤2	≤32	≥8	12(100%)	0(0%)

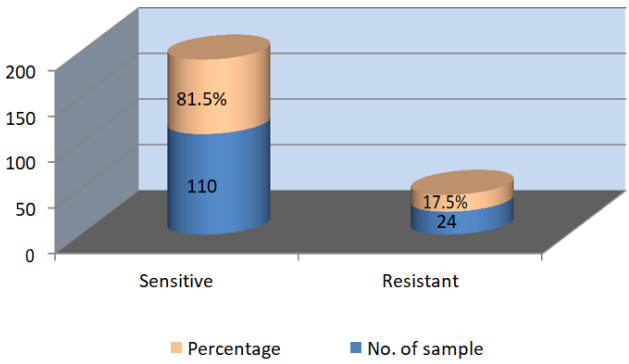

Figure 6: Showing data for different types of samples

In this study, we found 135 isolates of *Enterococcus* species where we did AST for various antibiotic drug panels through VITEK 2 Compact – fully automated and rapid ID/AST system. The results of AST for different drugs are shown in the table below.

Table 7: Data showing other antibiotics sensitivity to *Enterococcus* species

DRUGS	Enterococcus faecalis		E. faecium		Other Enterococcus species	
	S	R	S	R	S	R
Nitrofurantoin	62(83.8%)	12(16.2%)	49(100%)	0	12(100%)	0
Ampicillin	49(66.2%)	25(33.8%)	37(75.5%)	12(24.5%)	12(100%)	0
High level Gentamicin	37(50%)	37(50%)	12(24.5%)	37(75.5%)	12(100%)	0
Ciprofloxacin	74(100%)	0	49(100%)	0	12(100%)	0
Levofloxacin	74(100%)	0	49(100%)	0	12(100%)	0
Teicoplanin	74(100%)	0	49(100%)	0	12(100%)	0
Doxycycline	74(100%)	0	49(100%)	0	12(100%)	0
Linezolid	70(66.2%)	4(5.40%)	49(100%)	0	12(100%)	0

The implication of findings in hospital administration is that most of the hand hygiene procedure, personal hygiene condition should be taken in order to avoid this nosocomial infection. There were no remedial measures till now taken as the rate of VRE occurrence was known and further remedial measures can be taken in order to avoid the colonization of this VRE infections.

Discussion

Vancomycin-resistant *Enterococci* (VRE) causes serious infections that have historically shown to be difficult to treat, requiring combination therapy and careful monitoring of treatment-related damage. Despite the addition of novel antibiotics possessing VRE action to the treatment regimen, notable obstacles still exist. Clinicians who comprehend the mechanisms underlying the onset of resistance to VRE, the variation ingastrointestinal colonization, colonization resistance via microbiota, and the resistance method to presently accessible therapeutic options will find it easier to combat these difficult hospital-associated pathogens.

Antibiotics such as daptomycin and linezolid reduce VRE but maximizing their efficacy requires an understanding of their clinical role and resistance mechanisms. In the present study, we found 135 samples positive for *Enterococcus* species and 1542 were found negative. So, the prevalence for *Enterococcus* species in the whole sample was 8.05% which is similar to the study conducted in Kolkata in 2011 by **Chakraborthy et al.[13]** who found a 7.3% prevalence rate, and one other study conducted by **Phukanetal.[14]** who found 7.4% prevalence rate. It was discovered that the Vitek 2 automated system could accurately identify 93 *Enterococcus* species with the same accuracy as traditional biochemical tests. According to a study performed in Assam where they found 64.42% *E. faecalis* followed by 30.72% *E. faecium* and 1.08% found to be other species of *Enterococcus* while in our study we found that *E. faecalis* was 54.8% followed by *E. Faecium* 36.3% andother *Enterococcus* species was 8.9% which was near about to the previous case studied in north India.

In this study, we found that the most isolated *Enterococcus* species were detected from the age group 21-40 (45.9%) followed by 41-60 (36.3%), 0-20, and 61-80 (8.9%) while another study done by **Sivaradjy M et al.[10]**in 2021 says that the mean age of *Enterococcus* colonization and infection was 36 years. Some other studies by **Piezzi V et al.[15]** and **Sohn KM et al.[16]**found VRE colonization was common in the age group of 25 and 36 years.

The present study shows that the maximum number of patients were male adults 86(63.7%) and the rest were females 49(36.3%) who showed VRE positive in the sample while the other study conducted by **Sengupta M. et al.[5]** also found a prominent number of male patients 56% followed by females 44%. In the present study, the author finds out that the most frequent sample for a isolation of bacteria was urine sample 62(45.9%) followed by pus 25(18.5%), blood 24 (17.8%), and Foley's tip and other body fluid was 12(8.9%).

Another study done by **Sengupta M. et al.[5]** in 2023 found the most frequent sample for isolation of bacteria was urine at 60.11% followed by blood at 20.48%, pus at 18.33%, and body fluids at 1.08%which was most similar to our study.

Among the 135 positive samples from the present study processed for the AST with the Vitek-2 system which detects that Vancomycin-resistant *Enterococci* were 25 (18.5%) out of 135 samples and the other 110 samples were found sensitive to the antibiotic Vancomycin. So, the overall VRE detected from this study was 18.5%. Some other studies related to our present study conducted by the author **Das et al.[17]**in 2022 found the prevalence for VRE was 16.95% and another was **Deshpandeet al.[18]** in 2013 who also found a nearly similar prevalence rate for VRE19.59%which supports our study.

This data shows that there is a high rate of multiple drug resistance in our hospital which requires necessary attention for the treatment of the patients admitted in our hospital setting.

My study's limitation is that we have not used the broth dilution method, which is considered the reference method; instead, we have used the Vitek compact system for species determination and AST.

Suggestions required for future research include rapid diagnostic technique, identification of the biomarkers that help in early detection of VRE colonization for future therapeutics purposes, sampling of the environment in which investigation of the hospital environmental VRE and thus enhance the infection control procedures and lastly proper vaccination and their prophylactic measures should be taken. Thus, these are the areas that hold for improving detection, management, and prevention strategies of VRE infection in the hospital care settings.

Conclusion

After analyzing the context in healthcare studies, we discovered an increase in VRE bacteremia that is most likely related to patients' extended hospital stays and excessive antibiotic use.

The spread of VRE must be stopped by early detection, treatment, and preventive measures; failure to do so could have fatal side effects.

Screening patients who are vulnerable to VRE colonization is vitally important to actively begin proper measures of infection control which include: -

- Isolation of VRE-colonized patients
- Use of patient-specific equipment that strictly follows the guidelines of hand hygiene
- Lastly, cleaning the room thoroughly when the patient leaves the room.

Furthermore, it is crucial to abide by the guidelines for the antimicrobial stewardship program. These guidelines suggest the elimination of Vancomycin usage for medical procedures of antimicrobial prophylaxis which in turn shifts our focus to a susceptible narrow-spectrum antibiotic. This type of antibiotic is used in cases where the blood culture doesn't reveal the presence of beta-lactam-resistant Gram-positive bacteria, thereby avoiding the usage of Vancomycin empirically unless urgently required.[19]

Clinicians should take note of the rising trend in VRE and implement stringent antibiotic policies and infection control procedures to lower the VRE rate.

Ethical clearance: Taken from institutional ethical committee TMU Moradabad Ref no. TMU/ IEC Nov. 23/56 dated:24.11.23

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Conflict of interest: Nil

Reference

1. Bourdon N, Fines-Guyon M, Thiolet JM, Maugat S, Coignard B, Leclercq R et al. Changing trends in Vancomycin-resistant *Enterococci* in French hospitals. *Journal of Antimicrobial Chemotherapy*. 2011; 66(4):713–21.
2. Handwerger S, Raucher B, Altarac D, Monka J, Marchione S, Singh KV et al. Nosocomial outbreak due to *Enterococcus faecium* highly resistant to vancomycin, penicillin, and gentamicin. *Clin Infect Dis*. 1993; 16:750-5. (PMID-8329505)
3. Patel S, Preuss CV, Bernice F. Vancomycin. In: *StatPearls*. Treasure Island (FL): StatPearls Publishing; March 24, 2023.pmid-PMID: 29083794Bookshelf ID: NBK459263.
4. Saengsuwan P, Singkhamanan K, Madla S, Ingviya N, Romyasamit C. 2021. Molecular epidemiology of vancomycin-resistant Enterococcus faecium clinical isolates in a tertiary care hospital in southern Thailand: a retrospective study. *PeerJ*.2021;9:e11478
5. Sengupta M, Sarkar R, Sarkar S, Ghosh S, Banerjee P. Vancomycin and Linezolid-Resistant *Enterococcus* isolates from a tertiary care center in India. *Diagnostics*. 2023, 13:945.
6. Hsueh PR, Chen WH, Teng LJ, Luh KT. Nosocomial infections due to methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococci* at a university hospital in Taiwan. *Int J Antimicrob Agents*. 2005;26(1):43-9. (PMID-15975769).
7. Scagnelli M, Pellizer G, De-Lalla F, Emilio DA, Rassu M, Bragagnolo L, et al. Epidemiological analysis of vancomycin-resistant *Enterococci* in a large tertiary-care hospital in Northern Italy. *Eur J Clin Microbiol Infect Dis*. 2001; 20(9):609-16. (PMID-11714041).
8. Carmeli Y, Eliopoulos GM, Samore MH. Antecedent treatment with different antibiotic agents as a risk factor for vancomycin-resistant *Enterococcus*. *Emerg Infect Dis*. 2002; 8:802-7. (PMID-12141965).
9. National Nosocomial Infections Surveillance System. National Nosocomial Infections Surveillance (NNIS) System Report. *Am J Infect Control*. 2004; 32(8):470-85. (PMID-15573054).
10. Sivaradjy M, Gunalan A, Priyadarshi K, Madigubba H, Rajshekar D, Sastry AS. Increasing trend of Vancomycin resistant *Enterococci* bacteremia in a tertiary care hospital of south India: A three-year prospective study. *Indian J Crit Care Med*. 2021;25(8):881-5.
11. Sreeja S, Babu P R S, Prathab AG. The prevalence and the characterization of the *Enterococcus* species from various clinical samples in a tertiary care hospital. *J Clin Diagn Res*. 2012;6(9):1486-8. (PMID: 23285436).
12. Garcia-Garrote F, Cercenado E, Bouza E. Evaluation of a new system, VITEK 2, for identification and antimicrobial susceptibility testing of *Enterococci*. *J Clin Microbiol*. 2000;38(6):2108-11. (PMID: 10834961).

13. Chakraborthy A, Pal N, Sarkar S, Gupta M. Antibiotic resistance pattern of enterococci isolates from nosocomial infections in a tertiary care hospital in eastern India. Nat. Sci. Biol. Med. 2015:6; 394.

14. Phukan C, Lahkar M, Ranotkar S, Saikia KK. Emergence of VanA gene among vancomycin-resistant *Enterococci* in a tertiary care hospital of north-east India. *Indian J Med Res*. 2016; 143:357-61.

15. Piezzi V, Gasser M, Atkinson A, Kronenberg A et al. Increasing proportion of vancomycin resistance among *Enterococcal* bacteremia in Switzerland: a 6-year nationwide surveillance, 2013 to 2018. Euro surveill.2020;25(35):1900575.

16. Sohn KM. Duration of colonization and risk factors for prolonged carriage of vancomycin-resistant *Enterococci* after discharge from the hospital. *Int J Infect Dis* 2013;17(4): 240-46.

17. Das AK, Dudeja M, Kohli S, Ray P. Genotypic characterization of vancomycin-resistant *Enterococcus* causing urinary tract infection in Northern India. Indian *J Med Res*.2022; 155:423-31.

18. Deshpande VR, Karmarkar MG, Mehta PR. Prevalence of multidrug-resistant *Enterococci* in a tertiary care hospital in Mumbai, India. *J Infect dev Ctries*.2013;7:155-8.

19. Shrestha J, Zahra F, Cannady, Jr P. Antimicrobial Stewardship. Treasure Island (FL):2024.