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RESEARCH ARTICLE
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PREVALENCE OF VANCOMYCIN-RESISTANT ENTEROCOCCUS ISOLATED FROM VARIOUS CLINICAL SPECIMENS AND ITS ANTIMICROBIAL PROFILE AT TERTIARY CARE HOSPITAL, AHMEDABAD, GUJARAT, WESTERN INDIA

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Abstract:
Aims & Objective: Vancomycin-resistant Enterococci (VRE) represent a critical medical and public health concern due to their association with serious nosocomial infections and a high risk of mortality. Understanding the exact prevalence rate, virulence factors, and related risk factors among enterococci isolated from various clinical specimens is essential for controlling the spread of bacterial resistance and for epidemiological surveillance. This study aims to determine the prevalence of VRE and antimicrobial resistance profiles among enterococci isolates from clinical specimens at the institute.
Material & methods: This study was carried out on enterococci isolated from clinical specimens at the GCS Medical College, Hospital and Research Centre, from January 2024 to June 2025. Direct microscopic examination was done using wet film for urine specimens and Gram stain for other specimens, like pus and body fluids.. Inoculation was done on blood agar and MacConkey agar. Additionally, chocolate agar was inoculated for CSF specimens. Identification of enterococci by biochemical tests & antimicrobial susceptibility testing of enterococcal Isolates was done. Data was collected in an Excel sheet & analysed.
Result: On susceptibility testing, the prevalence of VRE was found to be 3.9 %. The maximum number of VRE isolates was from blood culture (52.38%), followed by Urine (28..57%) , tissue (9.53%), pus(4.76%), and body fluid (4.76%). Among VRE, 57.14% isolates were E. Faecium, followed by E. Faecalis (33.33%) and other enterococci (9.55%), respectively. Highest resistance was found for ampicillin, ampicillin-sulbactam, HLG, and teicoplanin, while the most sensitive was linezolid (95%).

Conclusion: A multi-disciplinary approach is urgently needed, including regular surveillance for the local epidemiology, early detection, and management, especially in the face of high-risk settings. Proper implementation of antimicrobial stewardship & infection control programs in the hospital is the best way to overcome the increasing trend of resistance.

Keywords: Vancomycin-resistant enterococci (VRE), prevalence, antimicrobial resistance, linezolid

Antimicrobial resistance (AMR) is currently one of the most significant public health issues worldwide, exacerbating the mortality risk associated with bacterial infections. Since its discovery, the rise of *vancomycin-resistant enterococci* (*VRE*) has become a major public health concern. [1] In 1984, group D streptococci were separated from the streptococci and were recognized as a distinct genus, which was named Enterococcus [2]. *Enterococci* are pairs or chains of facultatively anaerobic, gram-positive cocci that naturally inhabit the environment (soil, plants, etc.) and are an essential constituent of the normal human/animal gut flora.[3] There are currently around 50 different enterococcal species known. Human intestines often include *Enterococcus faecalis* and, to a lesser degree, *Enterococcus faecium.* The most common species in many food animals are *E. faecium*, *E. cecorum*, *E. faecalis*, and, to a lesser extent, *E. hirae*.[4] *E. faecalis* and *E. faecium* cause most enterococcal infections in humans, including hospital-acquired infections (HAI), and are highly resistant to multiple antibiotics.[5] *Enterococci* are opportunistic pathogens that cause serious and occasionally fatal infections. While most *E. faecium* infections are hospital-acquired, *E. faecalis* frequently causes infections in both the community and hospitals. The most common *enterococci*-related infections are endocarditis, urinary tract infections (UTI), intra-abdominal infections, and bloodstream infections (BSI). Rarely, they also cause pneumonia, septic arthritis, meningitis, and osteomyelitis.[6]

Enterococci are among the most common isolates, accounting for the second and third-highest nosocomial BSIs in the intensive care units (ICUs) in the US and Europe, respectively.[6] *Enterococci* become resistant to a variety of antimicrobials through intrinsic and acquired mechanisms.

Around 1.27 million deaths have been linked to infections caused by antibiotic-resistant bacteria. Among these, *Enterococcus faecalis* and *Enterococcus faecium* contribute to around 7.87% and 19.68% of the deaths, respectively.[7] *Enterococcus* species has great potential to attain antimicrobial resistance. Vancomycin-resistant enterococci (VRE) species have been increasing and are now of serious concern.[8,9] Vancomycin is a glycopeptide, and it works by binding to the D-Ala-D-Ala terminal of cell wall precursors, thereby inhibiting peptidoglycan synthesis.[9,10] VRE was first reported from Europe in 1988.[11]The first case from India was reported in 1999 from New Delhi.[9] Within a short span of time, it has become one of the predominant causes of nosocomial infections (Tripathi). The World Health Organization has categorized VRE as the most notorious bacteria in the "Global Priority List of Antibiotic-Resistant Bacteria."[9] VRE infection causes deterioration of the patient's condition with 65–70% mortality. Among the risk factors, the major ones associated with VRE bacteremia are prolonged hospitalization, prior exposure to antibiotics such as vancomycin, neutropenia, and renal insufficiency.[11]

However, isolates of *Enterococcus gallinarum* and *Enterococcus casseliflavus* exhibit intrinsic, low-level resistance to vancomycin with minimum inhibitory concentrations of up to 32 μg/mL [12]. On the other hand, *E. faecium*, *E. faecalis*, and numerous other *Enterococcus* species display acquired resistance to vancomycin [13]. Vancomycin resistance is obtained by these *Enterococci* via mutations and/or the gain of exogenous genetic material, which confers resistance [13]. Various genes such as *vanA*, *vanB*, *vanD*, *vanE*, *vanG*, and *vanL* have been proven to contribute towards vancomycin resistance in *Enterococci* [13].

Among the *enterococci* species, *E. faecium* has developed resistance to numerous antibiotic classes rather quickly. Ampicillin resistance in *E. faecium* reached a high degree. Since the 1980s, *E. faecium* has also developed resistance to fluoroquinolones, aminoglycosides, and glycopeptides, notably vancomycin. *E. faecalis* has also developed aminoglycoside resistance, although resistance to

ampicillin and vancomycin is much rarer than in *E. faecium*.[14] Alarmingly, *VRE* also exhibits resistance to tigecycline, linezolid, and daptomycin.

Centers for Disease Control and Prevention (CDC) in 2019 reported that this resistant infection caused 54,500 infections and 5400 deaths in hospitalized patients in the US in 2017. The crude mortality rates in the US and European nations range from 20 to 50 %.[6]There are no cost-associated studies available for *VRE* in India. The overall national data of the VRE infection rate is yet to be available from India due to a paucity of a cumulative data collection system. Understanding the exact prevalence rate, virulence factors, and monitoring antimicrobial resistance patterns among enterococci isolated from various clinical specimens is essential for controlling the spread of bacterial resistance and is important for epidemiological surveillance within the local healthcare settings. With this background, keep in mind that the study was planned with the objectives to find out the prevalence of VRE among enterococci clinical specimens because it is crucial to suspect enterococcal infection in the face of risk factors for better prevention and early management.

Better knowledge of these facts can help in formulating better control measures, which would help in lowering infection with VRE.[15]

MATERIALS AND METHODS

Study Site (Area)

This study was conducted at GCS Medical College, Hospital and Research Centre, Ahmedabad, Gujarat (a tertiary care hospital in western India). The study commenced after approval from the institutional ethical committee.

Study Design

This study is a retrospective cross-sectional study of the hospital database over a period of 1.5 years from January 2024 to June 2025.

Selection (Inclusion) criteria: Enterococci isolated from Clinical specimens, e.g., blood, urine, pus, wound discharge, CSF, and other body fluids (pleural and peritoneal) sent to the microbiology laboratory of the institute were included in the study.

Exclusion criteria:

Enterococcal isolates from stool, respiratory tract specimens, and vaginal swabs were excluded as colonizers.

Methodology:

An observational study was carried out in the microbiology department of a tertiary care hospital in Western India. A total of 18,823 samples were received over the duration of 1.5 years from January 2024 to June 2025 at the microbiology department were assessed.

Clinical isolates

The various clinical samples (specimens) like blood, urine, pleural fluid, cerebrospinal fluid, pus, ascetic fluid, etc., were collected from patients admitted in various wards like surgery, medicine, orthopaedics, gynaecology, paediatrics, etc., over the study duration of 1.5 years. The samples were processed for microscopy, culture, and sensitivity testing according to standardized laboratory protocols.

Specimen processing

Direct microscopic examination was done using wet film for urine specimens and Gram stain [16] for other specimens like pus and body fluids, looking for the presence of leukocytes and bacterial cells. Inoculation was done on blood agar [Fig. 1] and MacConkey agar. Additionally, chocolate agar was also inoculated for CSF specimens.

Fig. 1: Colonies of enterococci on blood agar

Identification of enterococci

Presumptive diagnosis of *enterococci* was based on their growth characteristics on sheep blood agar, MacConkey agar, Gram staining, i.e., Gram-positive cocci arranged in pairs at angles to each other, catalase-negative biochemical reaction, ability to grow in 6.5% NaCl, and bile esculin hydrolysis test [Fig. 2]. Identification up to *enterococcal spp.* level by carbohydrate fermentation tests using the following sugars – glucose, arabinose, mannitol, raffinose, lactose, sucrose, sorbitol, and trehalose (standard biochemical tests) [Fig. 3]. Hae-molysin production was detected in the strains of *E. faecalis* and *E.faecium* on sheep blood agar.

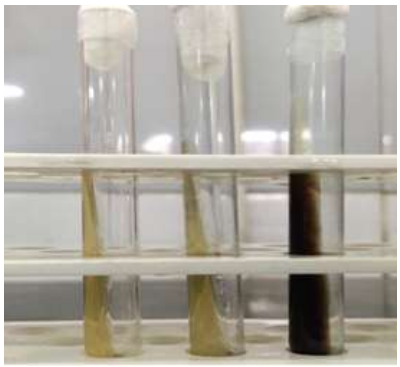


Fig. 2: Bile esculin agar test

Fig. 3- Arabinose, Raffinose fermentation test

Antimicrobial susceptibility testing of enterococcal Isolates

Antibiotic susceptibility testing- The susceptibility of the isolates against most commonly used antibiotics, such as ampicillin, ampicillin-sulbactam, gentamicin (HLG), levofloxacin, Vancomycin, teicoplanin, linezolid, and doxycycline, were evaluated by using the Kirby-Bauer disc diffusion method, and results were interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines. All the isolates first underwent initial Vancomycin Resistant *enterococci* screening by disc diffusion method using a disc of vancomycin (30µg) and later confirmed by determination of Minimal

inhibitory concentration (MIC) using Epsilometer test (E-strip) [Fig. 6, 7]. *Enterococcus faecalis* ATCC 51299 and *Enterococcus faecalis* ATCC 29212 were used as quality control strains.[17]

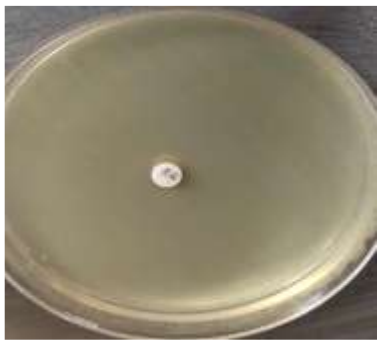


Fig. 4: Enterococcal strain showing resistance to vancomycin (30 mcg) disk

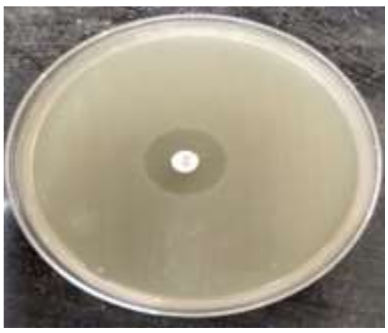


Fig. 5: Enterococcal strain showing susceptibility to vancomycin (30 mcg) disk

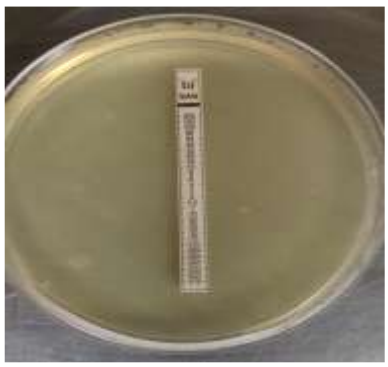
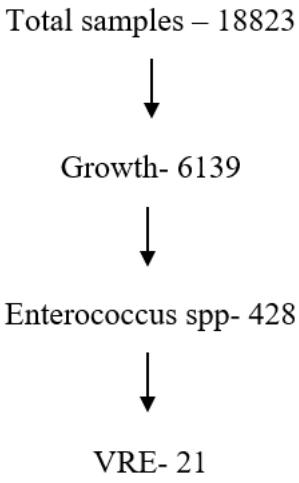


Fig. 6: Enterococcal strain showing resistance to vancomycin, MIC> 256 µg/mL

Fig. 7: Enterococcal strain showing susceptibility to vancomycin MIC=1.5 mg

Result

etween January 2024 and June 2025, a total of 18823 samples were received from patients with infection in different wards; among them, various micro-organisms were isolated in 6139 samples. Out of these 428 *enterococcal* strains isolated. Out of 428 *enterococcal* strains isolated from various clinical samples, 21 were VRE.



Among these 428 *enterococcal* strains isolated, 253 were *E.faecalis*, 161 were *E.faecium,* and 14 were other *Enterococcal spp*.

Among these 21 (4.9 %) VRE strains isolated, 7 were *E.faecalis*, 12 were *E.faecium,* and 2 were other *Enterococcal spp*.

Table 1- Prevalence of *enterococci* strains and VRE

***Enterococcal* strains**	**Enterococci strains (%)**	**VRE (%)**
E.faecalis	253 (59.11 %)	7 (33.33 %)
E.faecium	161 (37.62 %)	12 (57.14 %)
Enterococcal spp.	14 (3.27 %)	2 (9.53 %)
Total	**428**	**21**

Among these 428 *enterococcal* strains isolated, 223 were male & 205 were female, while in VRE, 9 were male & 12 were female**.**

Table 2- Sex wise Prevalence of *Enterococci Strains* & VRE

Sex	***Enterococcal* strains**	**VRE**
Male	223 (52.10 %)	9 (42.86 %)
Female	205 (47.90 %)	12 (57.14 %)

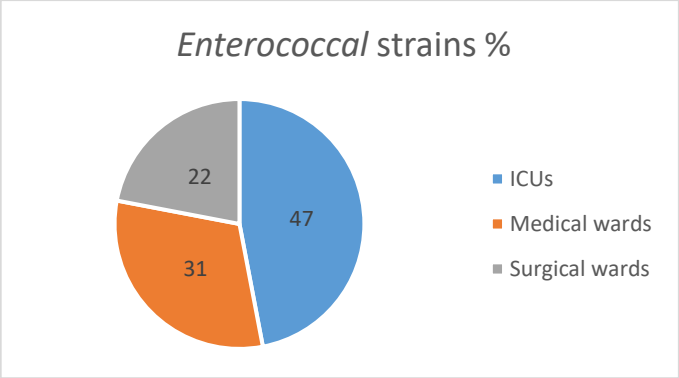
The maximum number of patients were in the 61-70 years age group at 24.06 % followed by the 51-60 years age group at 19.16%.

Table 3- Age-wise Prevalence of *Enterococci Strains* & VRE

Age Group	***Enterococcal* strains (%)**	**VRE (%)**
0-10	30 (7.01 %)	3 (14.28 %)
11-20	12 (2.80 %)	1 (4.76 %)
21-30	20 (4.67 %)	0 (0 %)
31-40	46 (10.75 %)	1 (4.76 %)
41-50	60 (14.03 %)	2 (9.52 %)
51-60	82 (19.16 %)	4 (19.06 %)
61-70	103 (24.06 %)	5 (23.81 %)
>70	75 (17.52)	5 (23.81 %)
Total	**428**	**21**

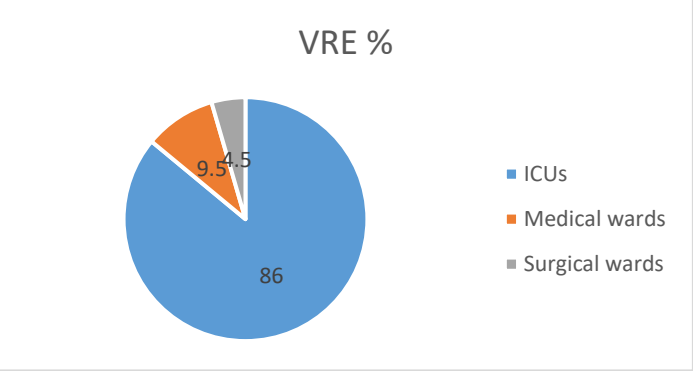
Among 428 *enterococcal* isolates, 201 were from the ICU, 132 from medical wards, and 95 from surgical wards.

Fig.8: Distribution of *enterococcal* isolates in different wards



While out of 21 VRE strains, 18 were isolated from the ICU, 2 were from the medical & 1 was from the surgical wards.

Fig.9: Distribution of VRE in different ward



The highest isolates of *Enterococci* were from urine (39.95 %), followed by pus & blood (24.77 % & 23.13 %)

Table 4- Prevalence of *enterococci* and VRE from various clinical samples

Sample type	***Enterococcal* strains (%)**	**VRE (%)**
Urine	171 (39.95 %)	6 (28.57 %)
Pus	106 (24.77 %)	1 (4.76 %)
Blood	99 (23.13 %)	11 (52.38 %)
Body Fluid	18 (4.21 %)	1 (4.76 %)
Tissue	34 (7.94 %)	2 (9.53 %)
Total	**428**	**21**

Antimicrobial susceptibility pattern of isolated *enterococci* has been given in Table- 5,6 & 7.

Table 5 - Antimicrobial susceptibility of isolated *enterococci*

Antimicrobial agent (µg)	**Sensitive (%)**	**Resistant (%)**
Ampicillin (10)	184 (42.99 %)	244 (57.01 %)
Ampicillin- sulbactum (10/10)	188 (43.93 %)	240 (56.07 %)
High-level gentamicin HLG (120)	141 (32.94 %)	278 (67.06 %)
Levofloxacin (5)	203 (47.43 %)	219 (52.57 %)
Doxycycline (30)	236 (55.14 %)	188 (44.86 %)
Linezolid (30	424 (99.07 %)	4 (0.03 %)
Vancomycin (30)	407 (95.09 %)	21 (4.91 %)
Teicoplanin (30)	407 (95.09 %)	21 (4.91 %)

Fig. 10: Enterococci antimicrobial susceptibility

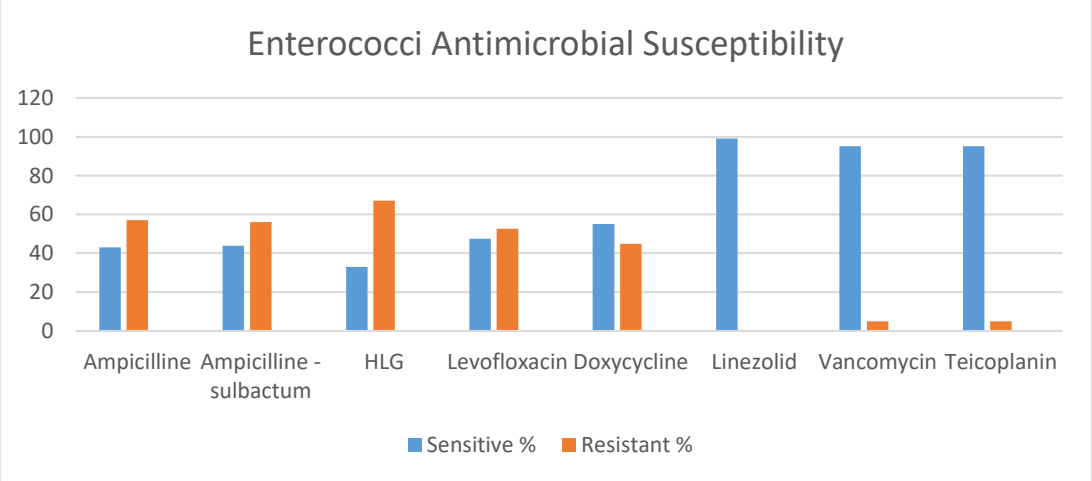


Table 6 - Antimicrobial susceptibility of VRE Out of 21 VRE

Antimicrobial agent (µg)	**Sensitive (%)**	**Resistant (%)**
Ampicillin (10)	0 (0)	21 (100)
Ampicillin- sulbactum (10/10)	0 (0)	21 (100)
High level gentamicin HLG (120)	0 (0)	21 (100)
Levofloxacin (5)	6 (29)	15 (71)
Doxycycline (30)	14 (67)	7 (33)
Linezolid (30)	20 (95)	1 (5)
Vancomycin (30)	0 (0)	21 (100)
Teicoplanin (30)	0 (0)	21 (100)

Fig. 11: VRE antimicrobial susceptibility

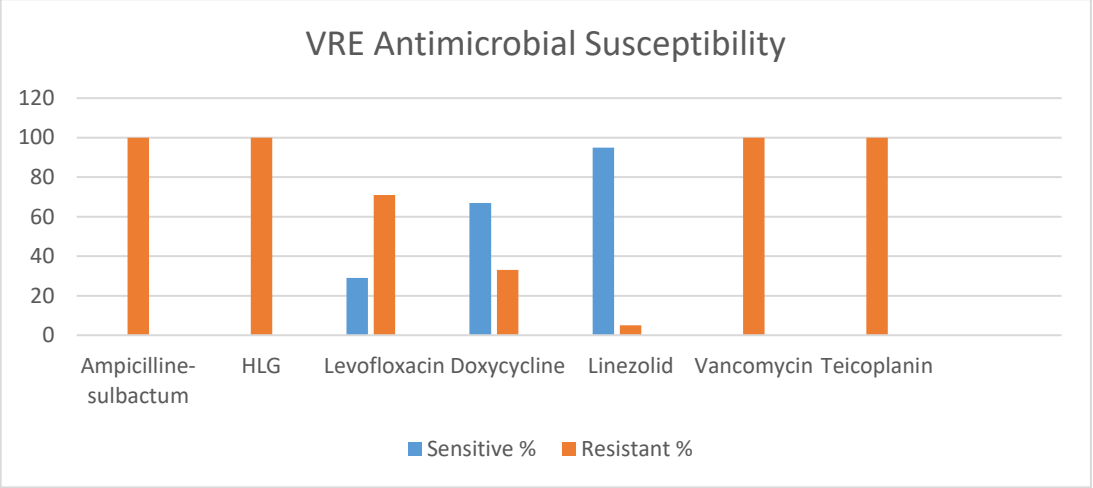
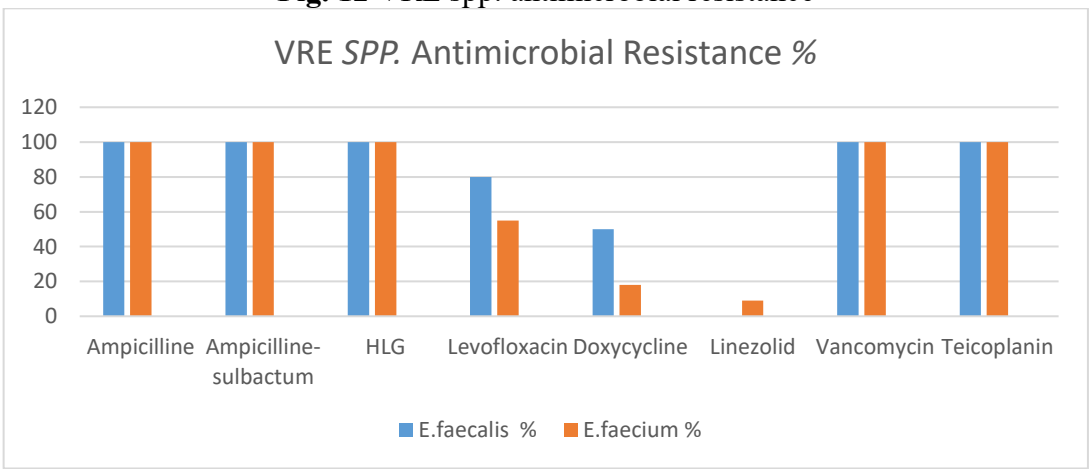


Table 7 - Antimicrobial resistance pattern in VRE spp.

Antimicrobial agent (µg)	*E.faecalis* (%)	*E.faecium* (%)
Ampicillin (10)	100	100
Ampicillin- sulbactum (10/10)	100	100
High level gentamicin HLG (120)	100	100
Levofloxacin (5)	80	55
Doxycycline (30)	50	18
Linezolid (30)	0	9
Vancomycin (30)	100	100
Teicoplanin (30)	100	100

Fig. 12 VRE spp. antimicrobial resistance



Discussion

Enterococcus, being considered an Innocuous commensal of the human and animal gastrointestinal tract for long periods of time, has come to hold the position of one of the most important nosocomial pathogens over the last few years, ranked as the second most common cause of healthcare-associated infections according to the National Healthcare Safety Network of the Centers for Disease Control and Prevention. Recently, enterococci have become organisms of increased interest amongst healthcare-associated infections owing not only to their ability to cause serious infections and to survive in hospital environments for long time periods, but also because of their intrinsic resistance to many antibiotics and increasing rates of acquired resistance to commonly used antibiotics.

Nosocomial transmission of enterococci can take place by direct or indirect modes, like the feco-oral route and invasive devices harbouring colonization by this flora. [16]

In this study, we isolated 428 consecutive enterococcal isolates from clinical specimens obtained from patients with a variety of enterococcal infections admitted to different wards and intensive care units of our institution. Study subjects were constituted by slightly more males (52.10 %) with a M: F ratio of 1.32:1. Some studies have also reported male preponderance [18,19], while female preponderance has also been reported. [20,21] The reason for the predominance of male patients may be that it is generally males who seek medical attention as compared to females, who tend to ignore their illnesses. Also, it depends upon the composition of study patients and the patient population seeking a particular healthcare facility. [22]

A CANWARD study from 2007 to 2013 revealed a 50% male and 50% female distribution with a 1:1 ratio of male: female with no gender predominance. [23] A prospective longitudinal study done in SICU to understand VRE also revealed no gender predominance.[24] Like our study, 55.06% of males and 44.94% of females were found to be infected, with little male predominance in a study done at ShriSathyaSai Medical College and Research Institute.[25]

The maximum number of patients with enterococcal infection belonged to the age group of 61-70 years at 24.06%. Like many studies where enterococcal infections were not commonly reported from paediatric patients, in our study, 6.01% of patients belonged to the ≤10 years age group. Unlike Yadav G *et al.* [26]**,** who reported the maximum patients (23.5%) in the 20-29 years age group and 11% of paediatric patients. In our study, VRE was maximum reported in > 60 years (23.81 %). Yilema A *et al.* [27] reported a median age of study subjects at 20 years and found that the age difference was not significantly associated with enterococcal infection, while paediatric patients were very high in number at 50%. This variation in age distribution of study participants can be explained by the varied composition of the study population and also the difference in frequency of infections at different body sites, which may have a certain age predilection.

Nosocomial UTI is the most common infection caused by these organisms. In our study, enterococcal isolates were obtained from a variety of clinical specimens, with the maximum (39.95%) from urine, followed by pus (24.77%) and then from blood culture (23.13 %). Tissue grew 7.94% of isolates, and 4.21% of strains were also isolated from body fluid. Various studies corroborated our findings with maximum clinical enterococcal isolation from urine samples, but the second most common infection noted in their study was wound infection, and the isolation rate from blood was lower, being reported as 2%, 7%, 13.6% and 3.74%.[28,29]

Unlike our study, the maximum number of isolates was obtained from urine (66%), with the study of Nautiyal S et al.[30] followed by pus (16.9%), other body fluids (7.5%), throat swab (7.5 %) & blood (1.8%), which is similar to the study of Maj Puneet Bhatt et al.[31]

A similar pattern was observed in VRE isolates. Similar results were found in a study done at a tertiary care hospital, Kolkata, by Mukherjee K. et al., with 80% enterococcus isolates from urine, followed by 16% from pus and 3.3% from blood.[32] In a study done by Jada S et al., the highest (40.30%) urine isolates were followed by pus and other body fluids (31.90%) and blood (18%). [25]

In case of VRE, in our study, 52.38 % isolates from blood culture, then 28.57 % from urine, 9.53 % from tissue & 4.76 % from both pus & body fluids.

In the present study, the maximum enterococcal species were isolated from the ICU (47%), then Medical wards (31%), and Surgical wards (22 %). Among VRE isolates, ICU (86 %) tops the list, followed by Medical wards (9.5%) and Surgical wards (4.5 %).

The CANWARD study done to understand VRE epidemiology in Canadian hospitals revealed 45% VRE isolates were from the medical ward, followed by 32.5% from the ICU setup, followed by 12.5% from surgical wards.[23]

In our study, *E. faecalis* was the predominant species being isolated at a rate of 59.11% followed by *E. faecium* (37.62 %). Similarly, S. Sreeja et al. [33] reported *E. faecalis* (76%) and *E. faecium* (24%). A similar species distribution was shown by many Indian studies, with isolation rates of *E. faecalis* ranging from 56% to 79.44% and that of *E. faecium* ranging from 20.56% to 34%. However, a few

reported *E. faecium* as the predominant species. However, a gradual increase in *E. faecium* isolation over that of *E. faecalis* can be explained by the selection of *E. faecium* after eliminating *E. faecalis,* as the earlier is more resistant to commonly used enterococcal antibiotics**.** [34.35]
In the present study, in the case of VRE *E. faecium* (57.14%) formed the major isolate, followed by *E. faecalis* (33.33%),
Risk factors for acquiring Vancomycin-resistant *enterococcal* infection are:

- Persons who have taken previous treatment with vancomycin and combinations of other antibiotics like penicillin and high-level gentamicin.
- Hospitalized patients who are on long-term antibiotic therapy.
- Persons with weak immunity, such as patients in intensive-care units, in transplant wards, patients with malignancy, and elderly patients, particularly in long-term care facilities.
- Surgical ward patients who have undergone abdominal or any other surgical procedure.
- Persons with central intravenous catheters or urinary catheters for a long duration.

VRE is transmitted among hospitalized patients most commonly by healthcare workers whose hands have inadvertently become contaminated, either from feces, urine, body fluids, or blood of a patient carrying the organism.
Infection control practice
In the present study, 57.01% isolates were resistant to Ampicillin, which is similar to the study of Mathur et al. [19]**,** who reported 66% isolates were resistant to Ampicillin. 67.06% isolates were resistant to Gentamicin which showed a drastic increase in resistance of the commonly used drugs, this type of resistance pattern with Gentamicin (77.7%) was also reported by Nautiyal S et al. & J. Parameswarappa et al. [20] In present study Linezolid showed the maximum susceptibility which is similar with the study of Chitnis S et al [21] as they found 100 per cent susceptibility of VRE to Linezolid.
Vancomycin resistance was noted in 4.9% of isolated *Enterococcus* strains. 95 % isolates were susceptible to linezolid. So, the choice of treatment for VRE isolates is linezolid.
In our study, the antibiogram of VRE reported 100% resistance against commonly used antimicrobial agents like Ampicillin and Gentamicin across various species of VRE. 71 % resistant to Levofloxacin & 33% resistant to Doxycycline. The most sensitive antimicrobial agent against VRE was Linezolid. In the present study, Teicoplanin is 100 % resistant in the VRE strain. So most of the strains of VRE were Van A phenotypes. VanB phenotypes are typically susceptible to Teicoplanin. In contrast to our study, a study done in a tertiary care hospital in Nigeria reported 100% resistance of VRE against Linezolid.[36] Similarly, a study done in Kolkata reported 70% resistance against Ampicillin and 100% sensitivity towards Linezolid.[37] Similar
findings were recorded in other studies.[38–41] Bhuyan B et al. recorded 79.4% resistance against penicillin, 67.9% against Ampicillin, and 69.6% against Levofloxacin. In addition, unlike our study 0% resistance was recorded against Linezolid.[42]
Documented VRE prevalence rates by Indian studies vary, as 8.72% from Pondicherry [43], 5.76% from Salem [44], 7.9% from Uttar Pradesh [45], and 7% from Kolkata [46]. The difference in the published rates of VRE infection may reflect differences in the infection control practices, antibiotic consumption policies, and cultural differences among health care personnel.

Conclusion

With a steady worldwide spread, VRE can be expected as a major challenge to healthcare authorities in the coming years. Vancomycin-resistant enterococci (VRE) are of both medical and public health importance, associated with serious multidrug-resistant infections and persistent colonization. Owing to its propensity to affect debilitated patients in critical care units, which are exposed to multiple empirical antimicrobials with frequent use of invasive devices, and its ability to survive in hospital environments for a longer duration, it becomes difficult to eradicate. Having limited therapeutic choices left, VRE can cause significant morbidity and mortality. Rational prescription of anti-

microbial agents, targeted VRE surveillance, and timely antibiogram in admitted cases is the need of the hour. A multi-disciplinary approach including regular surveillance for the local epidemiology, early detection and management especially in the face of high risk settings, implementation and strict compliance to infection control practices and antimicrobial stewardship, collaboration between treating clinician and microbiologists for timely de-escalation of empirical antimicrobials to avoid overuse is a need of time to prevent this infection and to curtail its intra- and inter-hospital, so the global transmission.

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