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Enterococcal infection rates from various clinical samples and phenotypic characterization of *Vancomycin-resistant enterococcus* (VRE) at tertiary care hospital

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ABSTRACT

Background: Enterococci are increasingly prevalent in both community and hospital-acquired infections, posing a significant challenge due to their resistance to common antibiotics, including vancomycin. Given the lack of specific prevalence data at our hospital, we conducted a study to isolate and characterize *Enterococcus* from various clinical samples and assess their resistance profiles, particularly to vancomycin, to guide more effective treatment strategies and improve patient outcomes.

Materials and Methods: All clinical samples such as blood, urine, pus, sputum, CSF and other fluids from the hospital were processed using standard bacteriological procedures, including Gram staining and culture on selective media. The enterococcal isolates were identified using conventional biochemical tests, including catalase test, bile esculin hydrolysis, and carbohydrate utilization tests. Antimicrobial susceptibility testing was performed according to CLSI guidelines.

Results: From a total of 12,013 clinical specimens, 50 enterococcal isolates were recovered. Among these, 34% were from blood, followed by 30% from urine, 16% from pus, 14% from fluids, 4% from sputum, as well as 2% from CSF. A total of 52% of the isolates were from indoor patients, and 62% were from women. *Enterococcus* faecalis was the second most common species, after *Enterococcus faecium*. The antibiotic susceptibility pattern demonstrated 100% susceptibility to linezolid. Resistance to vancomycin and teicoplanin was observed in 20% and 28% of isolates, correspondingly, with 70% of isolates exhibiting resistance to both vancomycin and teicoplanin.

Conclusions: The present investigation reveals a high rate of VRE (Vancomycin-resistance *enterococcus*) isolates in our hospital, with a majority of isolates showing resistance to both teicoplanin and vancomycin. The situation worsens due to the Van A phenotype and the multidrug resistance these medicines demonstrate, which leaves fewer therapeutic options for the treatment. This highlights how important it is to carry out routine surveillance to quickly identify and manage infections caused by *Vancomycin-resistant enterococcus* in both hospitals and the community.

1. Introduction

Group D streptococci, which included *Enterococcus*, were formerly recognized until 1984 as bacteria commonly found in the intestines of both animals and humans. They can be isolated from the skin, female genital tract, oropharynx, and gastrointestinal system, where they are most frequently found.[1,2] They are often detected in nosocomial infections as a component of a mixed flora.[1] Enterococci are

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known to cause hospital-acquired infections, particularly in critically ill or immunocompromised patients.[3] These infections can spread from patient to patient in healthcare facilities through direct contact with the hands of healthcare workers or indirectly through contaminated surfaces.[4] Enterococci can spread to normally sterile locations when host resistance is reduced, which can result in a range of diseases like bacteremia/sepsis, biliary tract infections, intra-abdominal abscesses, endocarditis, UTI (Urinary Tract Infection).[5] Enterococci are becoming more concerning as a pathogen due to its growing resistance to multiple drugs like vancomycin as well as ampicillin.[6] *Vancomycin-resistant enterococcus* (VRE) is worrying because there are limited treatment options available, resistance genes have the potential for transmission from *Enterococcus* to Staphylococcus aureus and various factors are promoting the spread of VRE and the growth of resistant strains.[7] In recent decades, they've become a significant hospital-acquired infection, resistant to many drugs, leading to increased illness and deaths among patients.[8] The majority of human infections usually occur by one of 2 species: *Enterococcus* faecalis or *Enterococcus faecium*. Evaluating the prevalence and evolving patterns of VRE infections is crucial for devising infection control strategies to reduce the morbidity and death linked with these infections, both within hospitals and in the community.

The purpose of this research is to estimate the predominance and to know the phenotypic properties of VRE in tertiary care hospital in Gujarat, India, given the growing concern for antibiotic resistance.

2. Materials and Methods

2.1. Study design

This prospective observational study was conducted over a two-year period, from October 2020 to October 2022, at the Department of Microbiology, GMERS Medical College and Hospital, Sola, Ahmedabad. Patients presenting with clinical symptoms of infection were enrolled and followed throughout their hospital stay. Various clinical samples were collected for analysis to observe the incidence of *Enterococcus* and the emergence of vancomycin-resistant strains over time.

2.2. Study population

The study population included patients attending both the inpatient and outpatient departments at GMERS Medical College and Hospital, Sola.

2.3. Study period

October 2020 to October 2022.

2.4. Type of samples

The samples collected included sputum, pus, cerebrospinal fluid (CSF), body fluids, urine, and blood, obtained from both outpatients and inpatients.

2.5. Type of sampling

Convenience sampling was used in this study.

2.6. Statistical analysis

After statistical analysis, the data are shown as percentages and numbers.

2.7. Processing of the samples

All clinical specimens (blood, urine, CSF, sputum, pus, and other fluids) were processed for bacterial isolation. Gram staining was performed for preliminary identification, followed by inoculation onto selective media such as MacConkey agar, bile esculin agar, and chocolate agar. Enterococcal isolates were identified based on conventional biochemical tests, including catalase test, bile esculin hydrolysis, growth in 6.5% NaCl, and carbohydrate fermentation tests. The species were confirmed based on their biochemical profiles.

2.8. Identification of Enterococcus species

The Gram stain revealed Gram-positive cocci arranged in pairs and short chains. The organism tested negative for catalase. On Chocolate agar, the colonies were small, cream-colored, smooth, and exhibited either alpha-hemolysis or were non-hemolytic, with an entire edge. On MacConkey agar, lactose-fermenting magenta-colored pinpoint colonies were observed. Additionally, growth on Bile esculin agar resulted in blackening of the medium, indicative of esculin hydrolysis. The organism also demonstrated the ability to survive in a 6.5% NaCl concentration, and it was capable of growing at 45°C.(Table 1)

2.9. Antibiotic susceptibility testing

Phenotypic resistance to antibiotics, including vancomycin and teicoplanin, was determined using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar, as recommended by CLSI guidelines (Clinical and Laboratory Standards Institute, M100, 31st edition, 2021).[9] Quality control for antimicrobial susceptibility testing (AST) was performed in accordance with CLSI guidelines. Standard ATCC (American Type Culture Collection) strains, such as *Enterococcus* faecalis ATCC 29212 for vancomycin, were used as control strains. All media, reagents, and antibiotic discs were tested for performance before use, and results were only accepted if the control strains performed within the recommended ranges. AST results were interpreted

Table 1: The differentiating features between *Enterococcus* species were observed as follows: [2]

Biochemical Test	***Enterococcus faecalis***	***Enterococcus faecium***	***Enterococcus durans***	***Enterococcus gallinarum***
Motility	Non-motile	Non-motile	Non-motile	Motile
Pigment production	None	None	None	None
Carbohydrate utilization	Sorbitol (fermenter)	Arabinose (fermenter)	Sucrose, mannitol (Non-fermenter)	Sucrose, Mannitol (fermenter)

based on CLSI breakpoints. Following antibiotics has been tested for *Enterococcus*: Levofloxacin 5μg, Tetracycline 30μg, Teicoplanin 30μg, Penicillin 10U, Ciprofloxacin 5μg, Vancomycin 30 μg, Erythromycin 15 μg, High-level Gentamicin 120 μg, Ampicillin 10 μg, Linezolid 30 μg, Nitrofurantoin 300 μg (only in urine samples). Additionally, isolates were screened for vancomycin resistance using vancomycin screen agar containing 6 μg/ml of vancomycin. The minimum inhibitory concentration (MIC) for vancomycin was determined using E-strips to confirm resistance. Enterococcal isolates with an MIC of ≥32 μg/ml were classified as *vancomycin-resistant enterococcus* (VRE). Van B phenotype is susceptible to teicoplanin but resistant to vancomycin, while the Van A phenotype is resistant to both of these substances. *Enterococcus gallinarum* is intrinsically resistant to teicoplanin and vancomycin, it shows Van C phenotype.

3. Results

The Microbiology department was the site of this investigation. Approximately 12,013 clinical samples were processed in total, comprising 3165 urine, 3180 blood, 3108 pus, 1562 sputum, 689 fluids, and 309 CSF. The majority of the enterococcal isolates had been from blood 0.5% (17/3180), followed by urine 0.5% (15/3165), pus 0.3% (8/3108), fluids 1.01% (7/689), sputum 0.12% (2/1562) and CSF 0.3% (1/309). The total prevalence rate of enterococcal isolates is 0.4% (50/12013). Of the isolates, 16 (32%) came from pediatric patients and 34 (68%) were from adult individuals. The isolation rate of approximately 62% (31/50) had been observed among female patients and 38% (19/50) among male individuals. Twelve enterococcal isolates (24%) came from intensive care units, 26(52%) from patients housed indoors, and twelve (24%) from patients outside. (Table 2)

We noted that the rate of isolation in patients of pediatrics was 30%, in gynecology was 22%, in medicine was 18%, and 10% in surgery patients. 36(72%) *Enterococcus faecium* is the most prevalent, next to 12(24%) *Enterococcus faecalis*, and also one species of *Enterococcus durans* from blood and *Enterococcus gallinarum* from ascitic fluid is isolated. [Table 3]

After analyzing the antibiotic susceptibility patterns, 92% of the isolates of E. faecalis displayed a high level of erythromycin resistance, while demonstrating a greater sensitivity to ampicillin at 58%. The *Enterococcus faecium* isolates have shown higher resistance rates to erythromycin (83%), ciprofloxacin (83%), levofloxacin (80%), and penicillin (72%). Our study found that multidrug resistance, which includes resistance to penicillin, tetracycline, fluoroquinolones, and aminoglycoside, is prevalent among the *Enterococcus faecium* isolates. A total of 60% HLAR-high-level aminoglycoside resistance is observed. The HLAR is noted 64% in *Enterococcus faecium* and 58% in *Enterococcus faecalis*. 20% (10/50) of Vancomycin resistant isolates have been identified. [Table 4]

3.1. Vancomycin screen agar results

In this study, all isolates were screened using vancomycin screen agar. Out of the 50 enterococcal isolates, 10 (20%) demonstrated growth on the *vancomycin-resistant enterococcus* (VRE) screening agar, indicating resistance to vancomycin. These results were consistent with the findings from the antimicrobial susceptibility testing, confirming the presence of vancomycin resistance in these isolates. The minimum inhibitory concentration (MIC) for vancomycin was determined using the E-strip method, with 10 isolates showing elevated MIC values (≥32 μg/ml), further confirming vancomycin resistance.

Out of the 10 VRE, 5 were isolated from blood samples, 3 from urine, and 2 from abdominal fluid and ascitic fluid. The majority of VRE was observed in hospitalized patients (90%). No mortality was observed in patients with *Vancomycin-resistant enterococcus* (VRE) infection in the present study. Out of 10 VRE, 7(19.44%) are Vancomycin resistant *Enterococcus faecium*. One isolate from *Enterococcus durans* and *Enterococcus gallinarum*. However, *Enterococcus gallinarum's* resistance to vancomycin and teicoplanin is intrinsic. [Table 5]

Linezolid was effective against all of the VRE isolates (100% sensitivity). Two isolates belong to the Van B phenotype 20% (2/10), which has a range of teicoplanin susceptible and vancomycin resistant. The remaining seven VRE isolates 70% (7/10), were of the Van A phenotype, which demonstrated resistance to both teicoplanin and vancomycin. One isolate is *Enterococcus gallinarum* which is intrinsically resistant to vancomycin and teicoplanin, so it belongs to Van C phenotype. [Table 6] The strains of *Enterococcus faecalis* and *Enterococcus faecium* are typically linked to the Van A & Van B phenotypes, which

Table 2: Distribution of enterococcal isolates on Age, Sex, and Facility types.

Total Samples	Enterococcal isolates	The age group of enterococcal isolates		Sex wise distribution of enterococcal isolates		Facility wise distribution of enterococcal isolates		
		Adult	Paediatric	Male	Female	OPD	IPD	ICU
12013	50 (0.4%)	34 (68%)	16 (32%)	19 (38%)	31 (62%)	12 (24%)	26 (52%)	12 (24%)

Table 3: Distribution of different *Enterococcus* species among the specimens**.**

Enterococcus Species	Urine	Pus	Blood	Fluids	Sputum	CSF	Total No
Enterococcus faecalis	5	5	1	1	0	0	12(24%)
Enterococcus faecium	10	3	15	5	2	1	36(72%)
Enterococcus durans	0	0	1	0	0	0	1(2%)
Enterococcus gallinarum	0	0	0	1	0	0	1(2%)
Total	15	8	17	7	2	1	50

Table 4: Antibiotic susceptibility pattern of total enterococcal isolates(n=50)

Antibiotic	Susceptible isolates (%)	Resistant isolates (%)
Penicillin	17(34%)	33(66%)
Ampicillin	20(40%)	30(60%)
Erythromycin	8(16%)	42(84%)
Tetracycline	14(28%)	36(72%)
Ciprofloxacin	9(18%)	41(82%)
Levofloxacin	9(18%)	41(82%)
High level Gentamycin	20(40%)	30(60%)
Vancomycin	40(80%)	10(20%)
Linezolid	50(100%)	0(0%)
Teicoplanin	36(72%)	14(28%)
Nitrofurantoin*	11(73%)	4(27%)

*Nitrofurantoin – only in 15 urine samples

Table 5: Pattern of vancomycin resistance among different species(n=10)

Species	Total isolates	Vancomycin resistant isolates
Enterococcus faecalis	12	1(8.33%)
Enterococcus faecium	36	7(19.44%)
Enterococcus durans	1	1(100%)
Enterococcus gallinarum	1	1(100%)
Total	50	10(20%)

Table 6: Phenotypic classification of VRE isolates (n=10)

Van phenotype	*Enterococcus faecium*	*Enterococcus faecalis*	*Enterococcus durans*	*Enterococcus gallinarum*	Total
Van A	5	1	1	0	7
Van B	2	0	0	0	2
Van C (intrinsic resistant)	0	0	0	1	1

are thought to be the greatest clinically significant.

4. Discussion

The present study observed a low prevalence rate of enterococcal isolates 0.4% (50 isolates from 12,013 samples), which contrasts with other studies reporting a prevalence of 1.46% to 85.8% across regions such as Ethiopia, Saudi Arabia, Lucknow, and Kolkata.[10–14] A significant proportion of isolates, 24% were obtained from indoor patients, and 52% from ICU settings. The high isolation rate from ICU patients could be attributed to invasive procedures, such as intravenous catheters, endotracheal intubation, urinary catheterization, comorbid conditions, prolonged hospitalization, and use of third-generation cephalosporins, all of which are common risk factors in ICU patients.

In this research, 68% of isolates (34 isolates from total of 50 enterococcal isolates) had been from adult individuals and 32% had been from the pediatric age group which having a similarity with the Acharya A. et al. research from Nepal[15] which reported an isolation rate of 30.5% from pediatric age group and a study by Ferede at al. from Ethiopia[11] which reported an isolation rate of 44% from pediatric age group. In adult patients in Medicine ward, comorbid conditions and recurrent infections were identified as risk factors, while malnutrition and prolonged hospitalization were noted in pediatric patients.

A higher isolation rate of 62% (31/50) was noted in female patients which correlates well with a study by Ferede et al[11] from Ethiopia. This could be due to the increased susceptibility of women to urinary tract infections (UTIs), a known risk factor for enterococcal infections. Given the association of *Enterococcus* with urinary flora, it is plausible that female anatomical factors contribute to a higher prevalence.

In the present research, 34% of the enterococcal isolates had been obtained from blood samples. This high percentage could be due to the bacteremic potential of *Enterococcus*, particularly in immunocompromised and critically ill patients, where bloodstream infections (BSIs) are common. The pathogen's resilience in the hospital environment and its association with healthcare interventions might explain the high isolation rate from blood. One enterococcal isolate was recovered from cerebrospinal fluid (CSF). This patient was a newborn admitted to the NICU with late-onset septicemia. Enterococcal infections in CSF in newborns are rare but can occur, particularly in immunocompromised neonates or those undergoing invasive procedures such as central line insertions. Further analysis of the patient's history and clinical presentation suggested that the infection might have been nosocomial in origin, linked to the hospital environment and prolonged NICU stay.

In this research, *Enterococcus faecium* is the most common species with 36 (72%), followed by *Enterococcus faecalis* with 12 (24%). These findings are comparable with studies from North India[11] which also reported higher isolation rates of *Enterococcus faecium* compared to E. faecalis. The predominance of E. faecium in our hospital could be due to its higher resistance to antibiotics, allowing it to survive in environments where antibiotic use is frequent. Multidrug resistance is resistant to penicillin, tetracycline, fluoroquinolones, and aminoglycosides, among the *Enterococcus faecium* isolates. While in most of the studies, *Enterococcus faecalis* is the predominant enterococcal species in studies from Nepal,[15] Saudi Arabia,[10] Lucknow,[16] Nagpur,[17] Uttar Pradesh[12] and central India.[18] In the present study, one species of *Enterococcus durans* from blood and *Enterococcus gallinarum* from ascitic fluid is isolated. Vittal P P et al. from south India[19] also reported *Enterococcus durans* from blood and *Enterococcus gallinarum* from miscellaneous sites.

The study demonstrated that all isolates were 100% susceptible to linezolid, which is consistent with global data. However, vancomycin resistance was observed in 20% of the isolates, with teicoplanin resistance in 28%. This may be due to the excessive or inappropriate use of vancomycin, particularly in hospitalized patients. 20% of isolates were *Vancomycin-resistant enterococcus* (VRE) in our study which is in correlation with the study from Arif D et al. from Uttar Pradesh[12] and Rahangdale VA et al. from Nagpur[17] that have reported a VRE isolation rate of 30% and 11.38% respectively. Subject population, antibiotic use, along geographical location all affect the occurrence of VRE.

The identification of 70% of isolates exhibiting resistance to both teicoplanin and vancomycin indicates the predominance of the Van A phenotype, which is notorious for multidrug resistance., 2 isolates belong to the Van B phenotype 20% (2/10) with vancomycin resistant and teicoplanin susceptible range. This research is comparable to that of Salem-bekhit et al. from Saudi Arabia[10] which reports 87.5% Van A phenotype and Ghoshal et al. from India[20] which reports 100% Van A phenotype. One isolate is *Enterococcus gallinarum* which is intrinsically resistant to vancomycin and teicoplanin, so it belongs to Van C phenotype.

The data from this study, including VRE prevalence and resistance patterns, are presented periodically at HIC meetings. These meetings play a crucial role in updating hospital policies on infection control and application of antibiotics, on the basis of surveillance data and findings from clinical studies.

In our hospital, the initiation of restricted antibiotics such as vancomycin is done in consultation with a physician. There is a clear policy for the use of restricted antibiotics to ensure judicious use and prevent the emergence of resistant strains. The hospital also has an antibiotic stewardship committee, which monitors the use of antibiotics across different departments, particularly in critical areas like the ICU. Regular audits are conducted by the infection control team to ensure de-escalation of vancomycin when appropriate, especially in ICU patients, to prevent unnecessary prolonged use.

5. Limitations of the Study

Although the antimicrobial susceptibility testing (AST) results indicated resistance patterns consistent with vancomycin resistance, molecular characterization of the Van A, Van B, and Van C phenotypes requires further genetic testing, such as polymerase chain reaction (PCR) or sequencing, to confirm the presence of specific resistance genes. This study relied solely on AST results to identify

VRE, without performing molecular testing to genotype the isolates, which limits the ability to definitively identify Van A, Van B, and Van C genotypes.

6. Conclusion

Enterococci are significant pathogens responsible for both hospital-acquired and community-acquired infections, with a growing impact on patient health. This study reveals geographical variations in the isolation and distribution of *Enterococcus* species, along with notable variation in vancomycin resistance. Our findings indicate a higher rate of vancomycin resistance (20%) in our region, with the Van A phenotype accounting for 70% of the vancomycin-resistant enterococci (VRE). Additionally, this study highlights the prevalence of multidrug resistance and high-level aminoglycoside resistance among enterococcal isolates, particularly in E. faecium. Due to geographical variation in enterococcal species distribution as well as Vancomycin resistance, routine surveillance to detect *Vancomycin-resistant enterococcus* (VRE) promptly should be implemented. Strict infection control measures, including judicious antibiotic use, proper containment practices, and the education of healthcare workers, are essential to reducing the morbidity and mortality associated with nosocomial VRE infections. Furthermore, antibiotic stewardship programs should be strengthened to monitor and regulate the use of antibiotics, particularly in critical care settings, to prevent the emergence of resistant strains.

7. Ethical Approval

The institutional ethical committee gave its approval to the project.

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8. Source of Funding

None.

9. Conflict of Interest

No conflicts of interest.

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