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Prevalence of Vancomycin Resistant Enterococcus in Various Clinical Specimens in Tertiary Care Hospital

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Abstract: ***Introduction*: Enterococcus is a commensal of the genitourinary tract and gastrointestinal. Recently, they have been recognised as pathogens of medical significance linked to infections acquired in both hospitals and the community. In patients with compromised immune systems, hospital-acquired infections are most commonly caused by VRE. Consequently, because of the high morbidity and fatality rate of VRE, treatment is crucial. Estimating the prevalence of VRE in diverse clinical specimens is the goal of this research. *Materials and methods*: A prospective observational investigation was performed in the Department of Microbiology, GMC, Ongole, AP from April 2023 to August 2023. Three hundred samples were taken from blood, urine, and OPD exudates in IP patients of every age and sexuality. AST and enterococcus were anticipated to be detected using the Kirby-Bauer disc diffusion method. MIC was estimated for every isolate of Enterococcus using the E test. *Results*: Out of 300 samples, 56 were Enterococcus isolates from 25 (45%) urine, 18(32%) blood, and 13(23%) exudates. Out of 56, 32(57%) E. faecalis, 24 (43%) E. faecium. VRE was detected from 04 (45%) urine, 03(33%) blood, and 02(22%) Exudates. *Conclusion*: In this study, 16% of VRE were found. Multidrug resistance has made Enterococcus a potential pathogen in modern times. Therefore, in order to prevent dissemination, an effective infection control programme, and routine surveillance must be implemented. Antibiotic stewardship must be properly followed to reduce antibiotic resistance (ABR).***

Keywords: VRE, HAI, CAI, Bile Esculin, E test, and MIC

1. Introduction

Enterococcus is a harmless commensal of the genitourinary tract and gastrointestinal tract. Recently, they have been recognised as pathogens of medical significance linked to infections acquired in both hospitals and the community. It is the second most frequent pathogen related with HAI, mainly in CAUTI and CLABSI.

Of the twenty enterococcus species, Enterococcus faecalis (85 percent-90 percent) and Enterococcus faecium (5–10%) are the most prevalent pathogens.

VRE infections have a greater effect on the healthcare system than vancomycin-susceptible enterococci as they are related with higher death rates, increased healthcare costs, and extended hospital stays.

Resistance to vancomycin When compared to vancomycin-resistant infections, E. faecium bacteremia has been related with a poor prognosis and a greater death rate. Bacteremia is caused by E. faecalis.

A rise in enterococcal infections is due to inappropriate antibiotic usage, extended hospital stays, invasive therapy, and widespread use of immunosuppressants [2]. It causes life-threatening infections like endocarditis, bacteremia, urinary tract infections, sepsis, intraabdominal abscess, and wound infections.

One of the medication alternatives for enterococci infections is vancomycin, which works by attaching to the terminal D-Ala-D-Ala pentapeptide and blocking peptidoglycan cross-linking, which weakens the integrity of the peptidoglycan layers and ultimately results in cell death.

Intrinsic resistance to numerous antibiotics, including cephalosporins, trimethoprim-sulfamethoxazole, and lincosamides, enterococci can also develop resistance to antibiotics through spontaneous chromosomal changes. High-level resistance to aminoglycosides, ampicillin resistance, and glycopeptide resistance are widely recognized cases of acquired antibiotic resistance in enterococci [9].

The production of various peptidoglycan precursors, which incorporate into the cell wall and have a lower binding affinity for glycopeptides, causes acquired resistance to glycopeptides.

"D-alanyl-D-alanine" is substituted with "D-alanyl-D-lactate" or "D-alanyl-D-serine" in strains that are resistant to glycopeptides.

As VRE are resistant to 1st-line antibiotics in hospital settings, daptomycin, tigecycline, and linezolid are some of the available therapeutic options. However, there has also been evidence of increasing resistance to these last-resort antibiotics. According to the WHO AWaRe classification, vancomycin belongs to the Watch group.

In patients with compromised immune systems, VRE is the most frequent cause of hospital-acquired infection. Due to the high morbidity and mortality of VRE, treatment is crucial.

Aim:

To study "the prevalence of vancomycin-resistant enterococcus in various clinical specimens in tertiary care hospitals.

Objectives:

1) To isolate and identify the enterococcus in various clinical specimens.
2) To detect the Antibiotic sensitivity testing of isolated pathogens.
3) To detect vancomycin resistance by E test.
4) To study the prevalence of VRE.

2. Materials and Methods

- **Study design:** Prospective observational study
- **Study period:** From April 2023 to" August 2023.
- **Study location:** Department of Microbiology, GMC, Ongole, Andhra Pradesh.
- **Sample Size:** 300 clinical samples.

Inclusion Criteria

1) Patients from OPD and IPD were taken.
2) Patients with all age groups and both sexes.
3) Samples like urine, blood, and exudates were collected.
4) Patients who have given informed consent.

Exclusion Criteria

1) Samples other than urine, blood, and exudates were excluded.
2) Pathogens other than enterococcus were excluded.
3) Patients who have not given consent.

Methodology

1) The current investigation was carried out at Government Medical College's Microbiology Department in Ongole, AP. A total of 300 samples, of which 137(57%) urine, 96(32%) blood, and 67(21%) exudates were collected under strict aseptic precautions from patients of every age group, both sexes and OPD, IPD from various Departments. Presumptive identification was by Macroscopic, microscopic examination. Samples have been inoculated on blood, MacConkey as well as nutrient agar, and then cultured aerobically at 37°C for 24 to 48hours.
2) Enterococcus isolates were identified based on colony morphology as:
   - Nutrient Agar: small, spherical, smooth, and opaque colonies.
   - Blood Agar: Smooth, gray, non-hemolytic colonies
   - MacConkey Agar: Small, magenta-coloured colonies.
3) Identification of enterococcus was done by Catalase, Bile esculin, Heat resistance test, Arginine hydrolysis, and sugar fermentation.
4) Using commercially available antimicrobial discs such as Ampicillin (10µg), ciprofloxacin (5µg), Gentamicin (120µg), linezolid (30µg), vancomycin (30µg), and teicoplanin (30µg), as well as control strains Enterococcus faecalis ATCC 29212 as the "positive control and Staphylococcus aureus ATCC 25923 as the negative control as per CLSI guidelines, antimicrobial susceptibility testing has been conducted by Kirby Bauer disc diffusion" approach on blood agar plates that have been covered with the turbidity equal to a 0.5 McFarland Standard. The E test was utilized to estimate the vancomycin MIC (Minimum Inhibitory Concentration) for all Enterococci isolates that showed resistance to the antibiotic employing the disc diffusion method.

3. Results

- A total 300 samples, of which 137(57%) urine, 96(32%) blood, 67(21%) exudates were collected (Table-1). 56(19%) Enterococcus were isolated. Among 244 (81%), 32(13%) were sterile and 212 (87%) were other pathogenic organisms.
- Identification of enterococcus was done by magenta pink colour colonies on macConkey agar (Fig-1), Catalase negative (Fig- 2), Bile esculin blackening (Fig- 3), Heat resistance survival at 60°C for 30min, Arginine hydrolysis shown deep purple colour. Mannitol, pyruvate, and sorbital fermentation was shown by E.faecalis, whereas as arabinose, mannitol fermentation was shown by E. faecium. (Fig-4)
- Out of 56, 25 (45%) enterococci isolated from urine, 18(32%) blood, and 13(23%) exudates (Fig- 5). Among 56 isolates 34(61%) were females ,22(39%) were males. (Fig- 6)
- Out of 56, 32(57%) were E. faecalis, and 24 (43%) were E. faecium (Fig- 7). Antibiotic sensitivity testing showed 100% sensitivity to linezolid, teicoplanin, and resistance to ciprofloxacin 46(82.1%), ampicillin 33 (58.9%), gentamycin 32 (57.1%) and vancomycin 18 (32.1%). (Fig- 8)
- MIC was done to all VRE samples by E test. Out of 18, 09(16%) were detected VRE. Out of 09, 04 (45%) from urine, 03(33%) blood, 02(22%) Exudates. (Fig-9)
- Out of 09(16%) VRE by E test detected-(MIC >128µg/ml)- in 05(56%) ,(MIC>96µg/ml)- in 03 (33%) , (MIC>64µg/ml)- in 01(11%) isolates.(Fig-10).

Table 1: Distribution of various samples collected

Sample	Total (300)
Urine	137
Blood	96
Exudates	67

Figure 1: MacConkey agar – showing magenta pink colour colonies

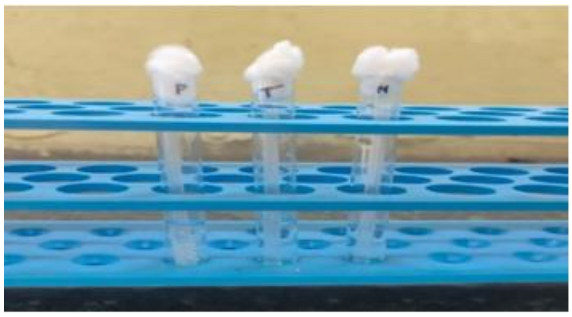

Figure 2: Catalase test

Figure 3: Bile esculin

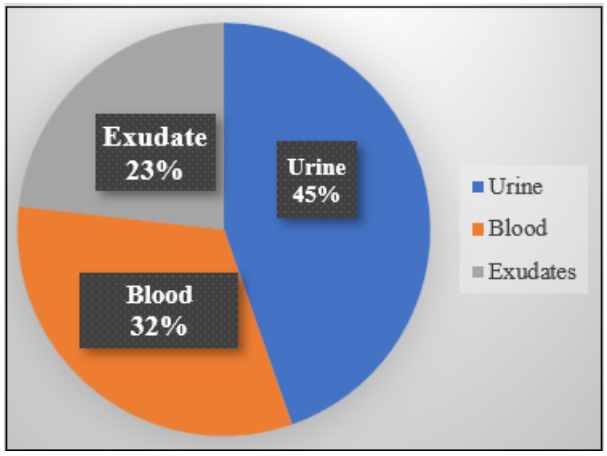

Figure 4: Distribution of enterococci from various clinical samples

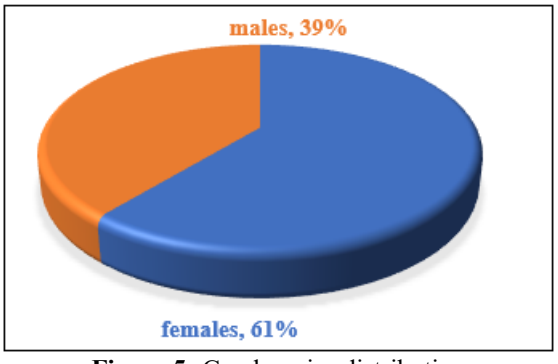

Figure 5: Gender wise distribution

Figure 6: Sugar fermentation

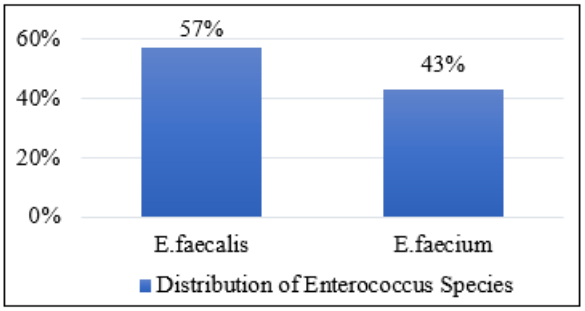

Figure 7: Distribution of enterococcus species

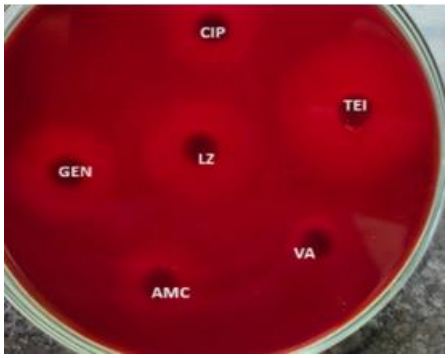

Figure 8: Antibiotic sensitivity pattern

Table 2: Antibiotic resistance pattern between different species of enterococci

S.NO	Antibiotics	E. faecalis (32)	E. faecium (24)	Total no. of Enterococci (56)
1.	Ampicillin	20(62.5%)	13(54.1%)	33(58.9%)
2.	Ciprofloxacin	26(81.2%)	20(83.3%)	46(82.1%)
3.	Gentamicin	25(78.1%)	07(29.1%)	32(57.1%)
4.	Vancomycin	06(18.7%)	12(50%)	18(32.1%)
5.	Linezolid	0	0	0
6.	Teicoplanin	0	0	0

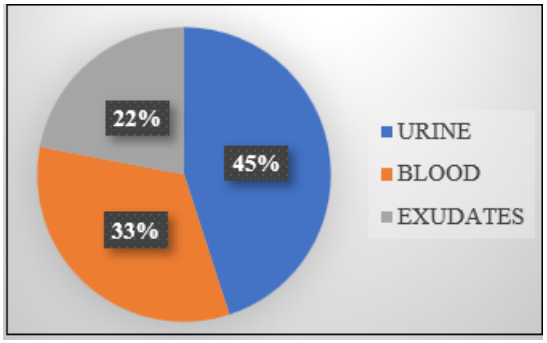

Figure 9: Distribution of VRE among various clinical samples

Figure 10: E test

4. Discussion

1) Enterococcus is a normal microbiota of GUT and GIT. It became one of the most common nosocomial pathogens linked to significant rates of morbidity and death.
2) It became most important due to the rise in resistance to multiple drugs like B lactams, aminoglycosides, and glycopeptides like vancomycin.
3) In patients with weakened immune systems, VRE is the primarily cause of hospital-acquired infections.
4) The current research was carried out to isolate, identify, and to find out the occurrence of VRE from numerous clinical samples. According to Maj Puneet Bhatt et al.'s investigation, the maximum number of enterococci isolates in our study came from 25 (45%) urine samples, which was comparable to the research performed by Nautiyal S et al., followed by 18 (32%), blood, and 13 (23%), exudates.
5) According to S. Sreeja et al., the most commonly cultured species in this research was E. faecalis, which was followed by E. faecium.
6) This study shows resistance to ampicillin in 33(58.9%) isolates, which is comparable to the research of Mathur et al. Vancomycin shown 18 (32.1%). Linezolid and teicoplanin shown 100% susceptibility similar to Chitnis S et al.
7) The current study's VRE prevalence of (16%) is comparable to that of Jafari-Sales A et al.'s study.
8) Among 09(16%) VRE, 04 (45%) from urine, 03(33%) blood, and 02(22%) Exudates, detected VRE.
9) E test of 09(16%) VRE detected (MIC >128µg/ml) in 05, (MIC>96µg/ml) in 03 , (MIC>64µg/ml) in 01 isolates.

5. Conclusion

The present study detected 16% VRE. Multidrug resistance has made Enterococcus a potential pathogen in modern times. Therefore, in order to prevent dissemination, an effective infection control programme, and routine surveillance must be implemented. Reducing antibiotic resistance, morbidity, and mortality in healthcare settings requires adherence to antimicrobial stewardship, thorough monitoring, and testing of VRE infections continuously.

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None

Conflict of Interest

None

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