Bmed-H
e-ISSN: 2582-7405
Transaction on Biomedical Engineering Applications and Healthcare
Vol-5 Issue-2, October 2024

Prevalence of Vancomycin-Resistant Enterococci at a Tertiary Care Hospital, Telangana

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Abstract

Background and Aim: *The second prevalent reason for urinary tract nosocomial infections and wounds is enterococci. Global outbreaks have been caused by multidrug resistance, particularly vancomycin-resistant enterococci, and their spread. The current investigation attempts to identify the distribution, susceptibility pattern, and prevalence of VRE (vancomycin-resistant enterococci) in diverse clinical isolates.*

Materials and Methods: *A cross-sectional, prospective study was carried out between December 2023 and May 2024 in the microbiology department of the tertiary care hospital. Approximately 76 distinct clinical isolates of enterococci were obtained from various clinical samples, including blood, pus, urine, and tissue fluids, sourced from both inpatient and outpatient departments, over the course of the study. The isolates were identified using both an antibiotic susceptibility test and a standard biochemical test in accordance with CLSI guidelines. The conventional techniques for identifying VRE were the Minimum Inhibitory Concentration test and the Kirby Bauer disc diffusion approach on Muller-Hinton agar.*

Results: *Of the 76 isolates, the majority (48.06%) came from urine, with blood cultures (33.08%) and pus swabs (5.66%) coming in second and third, respectively. According to susceptibility testing, 11.13% of people had VRE.*

Conclusion: *Patients with sepsis, wound infections, and urinary tract infections had increased rates of enterococcal infection. VRE was isolated from a variety of clinical isolates, and its prevalence (11.13%) suggests that MDR Enterococci have fewer options for antibiotic treatment. This emphasises the necessity of putting strong infection control measures in place, such as limiting the appropriate use of antibiotics, particularly vancomycin, in order to treat infections effectively and lower the mortality and normalcy rates related to hospital-acquired VRE infections.*

Keywords

Prevalence,Enterococci, Vancomycin Resistant Enterococci Anti Microbial Susceptibility, Nosocomial Infections.

INTRODUCTION

The Genus Enterococcus was described in 1980s as Gram-positive cocci and identified as a separate genus from *streptococci* as they showed agglutination with Group -D antisera in the Lancefield grouping of streptococci, 16sRNA sequencing, and DNA hybridization technique in 1984. Enterococci are Gram-positive, facultative anaerobes, often recognized as spectacles which are present in single, pairs, or short chains on smear. Despite being commensals of adult faeces, enterococci continue to be significant nosocomial pathogens[1].

They are commensals of the intestine, female genital tract, and oral cavity, including humans and animals. There are about 35 species known to exist, but 85–90% and 10-15% of human pathogens, respectively, are caused by *Enterococcus faecalis* as well as *Enterococcus faecium* [2]. These organisms could be differentiated from *Streptococci* by their capability to hydrolyze esculin in 40% bile, grow in the presence of 6.5% NaCl, and undergo proliferation in a wide temperature range of 5°C to 65°C[3]. They are also catalase-negative.

In the early 1990s, *Enterococci* were known to cause bacterial endocarditis. A review conducted on Nosocomial infections caused by bacteria revealed *Enterococci* were in 3rd place worldwide. These organisms gained recent importance due to the acquired glycopeptide resistance which not only limits the therapeutic drugs but also can get transferred to other organisms (ex: MRSA). Among nosocomial infections, *Enterococci* rank third in terms of bacteremia and second in terms of UTIs[4]. Among the hospital-associated Enterococcal infections, urinary tract infections remain the highest followed by surgical site infection and bacteremia. Enterococcal infections have been treated with drugs like glycopeptides (ex: vancomycin and teicoplanin), which act on the cell wall (ex: penicillin or ampicillin) when combined with an aminoglycoside (ex: gentamicin, streptomycin). However, the synergistic effects of combination therapy have failed due to the emergence of high-level resistance to vancomycin and aminoglycosides. First isolation of VER was done in the USA in the 1980s and in Europe in 1986. The species *E. faecium* which has gained resistance to vancomycin and penicillin has now become a therapeutically challenging organism at

e-ISSN: 2582-7405

hospitals[5]. Drug resistance in glycopeptides has occurred due to alteration of drug target sites from D-alanine-D-alanine to D-alanine-D-lactate. For isolates obtained from blood cultures and heart valves, CLSI has recommended the screening of enterococci with gentamicin and streptomycin respectively. The emerging multi-drug resistance especially vancomycin resistance among Enterococci and its spread has been responsible for many hospital outbreaks world with *E.faecium* and *E.faecalis* becoming the species of international concern. Among the risk factors, enterococcal infections are known to spread through previous/prolonged antibiotic usage, acquired glycopeptide resistance, surgical infections, catheterizations, immunocompromised states like HIV, cancers, diabetic patients, previous/prolonged hospital stays.

AIM OF THE STUDY

This "study purpose was to ascertain the prevalence, antimicrobial susceptibility patterns, and associated factors of VRE in a variety of clinical specimens from patients" receiving tertiary care.

MATERIALS AND METHODS

Six months of this prospective study, from December 2023 to May 2024, saw the collection of 76 distinct clinical specimens that were not duplicates from various clinical isolates.

Inclusion Criteria

1. Various clinical isolates from patients admitted to different hospital wards, including blood, urine, CSF, pleural fluid, pus, and ascitic fluid.
2. Physicians requested clinical isolates for culture and anti-microbial susceptibility.

Exclusion Criteria

Samples obtained from sputum, stool, throat swab, and vaginal swabs have been excluded from the study.

BACTERIAL ISOLATION AND IDENTIFICATION

Figure 1. Demonstration of *Enterococci* from urinary isolate

Received clinical specimens like blood, urine, CSF, pleural fluid, pus, and ascitic fluid from patients who have been admitted in several wards of the hospital and have been then "cultured on Blood and MacConkey agar. Subsequently, the isolates were found to be devoid of Catalase, Gram-positive cocci organized in pairs and chains" at a right angle, colonies that were either non-hemolytic or alpha-hemolytic on 5% sheep blood agar, and they tested positive for bile aesculin hydrolysis.

Bile Esculin Hydrolysis Test

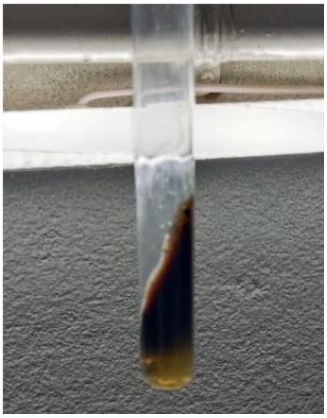

Figure 2. Bile Esculin Hydrolysis Test

Using a sterile inoculating needle, a well-isolated colony is removed from a culture plate after 18 to 24 hours. The light inoculum that was selected from the culture plate was streaked across the slant surface of the bile esculin agar tubes to initiate the inoculation process. To identify enterococcus and *S. bovis*, 40% bile is used. A 10µl calibrated loopful of a 0.5 McFarland standard suspension made in peptone water was streaked down the tubes. To guarantee sufficient aeration, the test tube caps were left undone. The tubes underwent an aerobic overnight incubation at 37°C, during which the color change was noted. Blackening of the medium in a ferric ammonium citrate-containing medium indicated a positive tube test. No color change indicates a negative tube test.

Heat Tolerance Test

To attain a stationary phase of growth, isolates were cultivated on nutrient broth and incubated overnight at 37°C for an additional 24 hours. Prior to heating, viable counts were ascertained through a series of 10-fold dilutions, and 0.1-milliliter volumes were cultured on blood agar plates. A water bath was used to incubate aliquots (0.1 ml) of each culture for 30 mins at the temp of 60°C, 10 mins at the temp of 65°C, 10 minutes at 71°C, and 10 minutes at 80°C. The 0.9 ml sterile water was then added. They kept an eye on the temperature. Following the exposure to the above temperatures, there was a rapid cooling period. On blood agar plates, 0.1 millilitres of the isolate was subcultured. For enrichment, the remaining 0.9ml has been then added to 9 ml of nutrient broth. For 48 hours, agar plates were incubated at 37°C. By calculating the log reduction in the number of viable organisms before and after exposure to heat, the killing effect was measured. *Streptococcus bovis* was heat labile, whereas *enterococci* were heat tolerant.

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e-ISSN: 2582-7405

Antimicrobial Susceptibility Testing

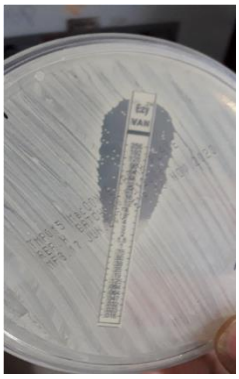

Figure 3. Testing of Vancomycin resistance by E-strip method

As recommended by CLSI guidelines, modified Kirby Bauer disk diffusion has been applied to all of the isolates of *enterococci*. In accordance with CLSI guidelines, antibiotic susceptibility testing has been performed carried out by utilizing the Kirby-Bauer disk diffusion approach with antibiotic disks on Mueller Hinton Agar. On Mueller Hinton agar plates, bacterial suspensions that met the 0.5 McFarland turbidity standard were made and lawn cultured. The following antibiotic discs were placed: Ampicillin (25µg), Norfloxacin (10µg), Nitrofurantoin (300µg), Clindamycin (10µg) (Himedia), Minocycline (30µg), Chloramphenicol (30 µg), Vancomycin (30µg), Erythromycin (30µg), Tetracycline (30µg), and Ampicillin (25µg). In accordance with guidelines provided by the Clinical and Laboratory Standards Institute (CLSI), the results were interpreted[6]. If the inhibition zone diameter surrounding the vancomycin disc was less than 14 mm or more than 17 mm, the isolates were deemed susceptible to vancomycin[7]. Utilised as the quality control strain was *Enterococcus faecalis* (ATCC 29212).

RESULTS

Of the 76 isolates, the majority (48.06%) came from urine, with blood cultures (33.08%) and pus swabs (5.66%) coming in second and third, respectively. According to susceptibility testing, 11.13% of people had VRE.

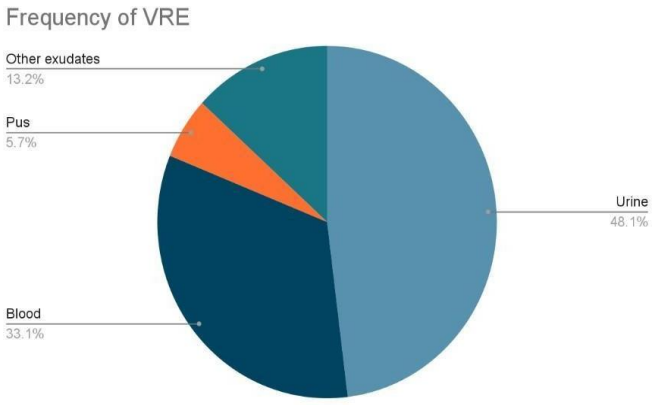

Figure 4. Distribution of VRE among various clinical specimens

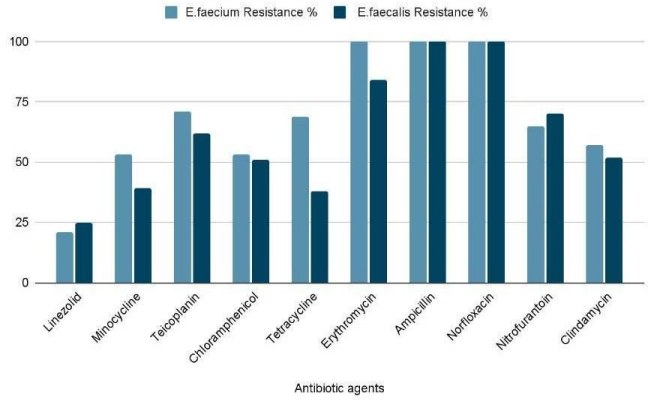

Figure 5. Antibiotic susceptibility pattern of VRE

DISCUSSION

The present study revealed the VRE prevalence was to be 11.13%. Melese A. et al. conducted "a systematic review and meta-analysis that included 20 studies from PubMed, EMBASE, Google Scholar, African Journals Online (AJOL), and other sources[8]. The pooled prevalence of VRE was found to be 14.8%. In a study conducted at a tertiary care center in Northern India, the prevalence of VRE was 7.9%.. It was discovered that the combined frequency of VRE was 14.8%. 7.9% of patients had VRE in a study done at a tertiary care facility in Northern India[7]. Urine produced the greatest number of *enterococcus* isolates (48.1%), followed by blood cultures (33.13%), pus swabs (5.7%), and other sources (13.2%). The highest percentage of isolates from urine (48.1%) in our investigation matched that of a prior investigation by Mukherjee K. et al. at a tertiary care hospital in Kolkata[9]. In that investigation, urine accounted for 80% of the isolates, with pus accounting for 16% and blood for 3.3%. In a study by Jada S et al., the percentage of isolates from urine was highest (40.30%), followed by pus and other bodily fluids (31.90%) and blood (18%)[10]. Zhanel et al. conducted a study at the University of Manitoba, USA, focusing solely on urinary isolates[11]. According to the study, 2.4% of VRE isolates were resistant to chloramphenicol, 47.6% to gentamicin, 85.8% to ampicillin, and 3% to linezolid. In contrast to their findings, we found that our VRE isolates were 100% susceptible to ampicillin and norfloxacin.

CONCLUSION

This increased prevalence of VRE in the hospital setting is largely due to inappropriate sanitation practices and the careless use of broad spectrum antimicrobial agents. Due to increased treatment costs, the high frequency of VRE is causing nosocomial infections and patient morbidity[12]. According to this study, VRE is most susceptible to ampicillin and norfloxacin. This study emphasizes how important it is to run regular surveillance programs in order to quickly identify VRE in communities and hospitals. This emphasizes the necessity of putting strict infection control measures in place, such as appropriate containment, efficient

treatment of VRE infections, and the sensible utilization of antibiotics—especially limiting the utilization of vancomycin.

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