

DETECTION OF VANCOMYCIN RESISTANT ENTEROCOCCI IN TERTIARY CARE HOSPITAL

Shiv Kumar Mehi¹, Md Shakir Alam¹, Ranjan Kumar¹, Amit Kumar²

¹Tutor, Department of Microbiology, JLNMCH, Bhagalpur, Bihar, India.

²Associate Professor, Department of Microbiology, JLNMCH, Bhagalpur, Bihar, India.

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Corresponding Author:

Dr. Md Shakir Alam,

Email: drshakir44all@gmail.com

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Abstract

Background: The genus *Enterococcus* consists of Gram-positive, facultatively anaerobic organisms that are spectacle shaped and may appear on smear in short chains, in pairs or as single cells. Enterococci, though commensals in adult feces are essential nosocomial pathogens. The emergence of multidrug resistant Enterococci, especially Vancomycin Resistant Enterococci (VRE), and its spread has caused the occurrence of many hospitals out breaks worldwide. In India, the prevalence of VRE has been reported as 8%, 5.5% and 23% in New Delhi, Chandigarh, and Mumbai, respectively, all of vanB phenotype. Enterococci have emerged as the leading causes of multiple drug resistant hospital-acquired pathogens especially with the emergence of glycopeptide-resistant *Enterococcus* (GRE) species. **Materials and Methods:** This study was conducted during the period from January 2022 to December 2022 at the Department of Microbiology, Darbhanga Medical College, Darbhanga. During the study a total of about 100 non duplicate clinical isolates of Enterococci were collected from different clinical samples. Antibiotic susceptibility testing was done by Kirby Bauer disc diffusion method. Vancomycin resistance was detected using Vancomycin screen agar and Hicomb E strip method. Data collected were entered in Microsoft Excel and analysed using statistical analysis software Statistical Package for Social Services (SPSS) v.16. **Result:** Prevalence of resistance was against Penicillin (85%), followed by Ciprofloxacin (67%) and Vancomycin (65%). On the other hand, Doxycycline was found to be sensitive for 10% of isolates, followed by Chloramphenicol (45%) and High level Gentamicin (33%). Out of the 100 enterococcal isolates, 77 isolates were found to be multi drug resistant (resistant to three or more antibiotics). **Conclusion:** This study emphasises the need for conducting frequent surveillance. Programmes for prompt identification of VRE in hospitals and community.

INTRODUCTION

The genus *Enterococcus* consists of Gram-positive, facultatively anaerobic organisms that are spectacle shaped and may appear on smear in short chains, in pairs or as single cells.^[1] Enterococci, though commensals in adult feces are essential nosocomial pathogens. Enterococcal infections may of at least 12 species including *Enterococcus faecalis*, *E. faecium*, *E. durans*, *E. avium*, *E. casseliflavus*, *E. gallinarum*, *E. hirae*, *E. malodoratus*, *E. mundtii*, *E. pseudoavium*, *E. raffinosus*, and *E. solitarius*.^[2] Among enterococcal species, *E. faecalis* and *E. faecium* are the two major human pathogens accounting for 85-89% and 10-15% of all enterococcal infections, respectively.^[3] Prior to the 1990s also, enterococci have been recognized as an important cause of bacterial endocarditis for almost

a century.^[4] However, recently they are recognized as a cause of nosocomial infection and "superinfection" in patients receiving antimicrobial agents. The most common Enterococci-associated nosocomial infections are of the urinary tract, followed by surgical site infections and bacteremia.^[5] The intrinsic antibiotic resistance pattern of Enterococci, along with their promiscuity in acquisition and dissemination of genetically versatile antibiotic resistance elements, presents serious challenges to the treatment of enterococcal infections. Infections by Enterococci have traditionally been treated with cell wall active agents (e.g., penicillin or ampicillin) in combination with an aminoglycoside (streptomycin/gentamicin),^[6] More ever, emergence of high level aminoglycoside resistance (HLAR), lactam antibiotics and to vancomycin by some strains has led to failure of synergistic effects of combination therapy.

Vancomycin is an effective antimicrobial for treating infection caused by gram positive organisms. Gram positive isolates are often routinely tested for vancomycin susceptibility. In the 1970s, hospital-associated enterococcal infections in the United States were mainly due to *E. faecalis*. More recently, *E. faecium* has emerged as therapeutically challenging organism because of its resistance to vancomycin and penicillin. These VRE isolates also have a high level of resistance to aminoglycosides. Resistance to glycopeptides is mediated by alteration of the drug target from D-alanine-D-alanine to D-alanine-D-lactate. The CLSI recommends screening of enterococci for high level aminoglycoside resistance with both gentamicin and streptomycin isolated from blood cultures or specimens such as heart valve tissue.^[7] The emergence of multidrug resistant Enterococci, especially Vancomycin Resistant Enterococci (VRE), and its spread has caused the occurrence of many hospitals out breaks worldwide. In the United States, vancomycin-resistance Enterococcus faecium accounted for 4 per cent of healthcare-associated infections. It is the second most common pathogen causing mortality and morbidity and the 3rd leading cause of hospital acquired blood stream infection.^[8] The prevalence in Asian countries is decreased and probably due to recent emergence of this resistance in this continent and only a handful of studies to document. In India, the prevalence of VRE has been reported as 8%, 5.5% and 23% in New Delhi, Chandigarh, and Mumbai, respectively, all of vanB phenotype.^[9] Enterococci have emerged as the leading causes of multiple drug resistant hospital-acquired pathogens especially with the emergence of glycopeptide-resistant Enterococcus (GRE) species.

MATERIALS AND METHODS

This study was conducted during the period from January 2022 to December 2022 at the Department of Microbiology, Darbhanga Medical College, Darbhanga. During the study a total of about 100 non duplicate clinical isolates of Enterococci were collected from different clinical samples like clinical specimens such as urine, blood, pus, tissue fluids obtained from both in-patient and out-patient departments of Darbhanga Medical College. The isolates were identified by standard biochemical tests. Antibiotic susceptibility testing was done by Kirby Bauer disc diffusion method. Vancomycin resistance was detected using Vancomycin screen agar and Hicomb E strip method.

Statistical Analysis

Data collected were entered in Microsoft Excel and analysed using statistical analysis software Statistical Package for Social Services (SPSS) v.16. Appropriate descriptive and inferential statistics were calculated. P values <0.05 were statistically significant.

RESULTS

Among the 100 enterococcal isolates, 65 isolates were from male patients and 35 from female patients. Most of the isolates (29%) were from patients aged between 16 and 30 years. The mean age of the patients was found to be 35.6 years with a SD of 21.5 years. The age of the patients ranged from a minimum of 2 years to a maximum age of 84 years.

Out of the 100 samples, 75 samples were collected from inpatients and remaining 25 from outpatients. Enterococci were commonly isolated from urine (90%), followed by sputum (7%), ascitic fluid (1%), pus from leg ulcer (1%) and vaginal swab (1%) [Table 1].

The isolates were identified to be Enterococcus faecalis (95%) and Enterococcus faecium (5%). All the seven sputum isolates and pus isolates were found to be *E. faecalis* whereas the ascitic fluid isolate and vaginal swab isolate was found to be *E. faecium*.

The [Table 2] shows the antibiotic susceptibility pattern of the enterococcal isolates by Kirby bauer disc diffusion method on Mueller Hinton agar according to CLSI guideline. Highest prevalence of resistance was against Penicillin (85%), followed by Ciprofloxacin (67%) and Vancomycin (65%). On the other hand, Doxycycline was found to be sensitive for 10% of isolates, followed by Chloramphenicol (45%) and High level Gentamicin (33%).

Out of the 100 enterococcal isolates, 77 isolates were found to be multi drug resistant (resistant to three or more antibiotics).

Enterococcus species have been recognised as a pathogen causing diseases like bacteremia, endocarditis, complicated urinary tract infections, intra abdominal infections, pelvic infections, wound and soft tissue infections etc. VRE has become an important nosocomial pathogen because of its rapid spread, high mortality rates associated with infections, limited option for treatment, and the possibility of transferring vancomycin resistance genes to other more virulent and more prevalent pathogens such as *Staphylococcus aureus*. This study was conducted to detect prevalence of vancomycin resistant enterococci in clinical isolates by phenotypic and genotypic methods.

Table 1: Distribution of the Enterococcal isolates in different clinical samples

Sample	Percentage
Urine	90
Sputum	7

Pus	1
Vaginal swab	1
Ascitic fluid	1

Table 2: Antimicrobial sensitivity pattern of the Enterococcal isolates by disc diffusion method (N=100)

Antibiotic	Sensitive isolates (%)	Resistant isolates (%)
Penicillin	15	85
Tetracycline	70	30
Ciprofloxacin	33	67
Chloramphenicol	45	55
Doxycycline	60	40
High level Gentamycin	23	77
Vancomycin	35	65

DISCUSSION

A total of 100 enterococcal isolates were collected from 969 culture positive samples received over one year period.

95% of the isolates were identified to be *E. faecalis* and 5 % was *E. faecium*. Both these species are significantly associated with clinical disease, Parameswarappa et al., in their study have found *E. faecalis* to be the predominant isolate followed by *E. faecium*. Chakraborty et al., also reported *E. faecalis* as the predominant Enterococcal species (80%-90%) in their study followed by *E. faecium* (5%-15%).^[10] Several species of enterococci are currently recognized, but generally 95% of enterococcal infections are caused by *E. faecalis* and 5% are caused by *E. faecium*. Although a few studies have documented an increase in the prevalence of *E. faecium*, in this study the prevalence of this species was considerable low. *E. faecium* infections have been found to be more resistant to penicillin and aminoglycosides which is attributed to the production of enzyme 6-acetyl transferase and more penicillin binding proteins.

Resistance to many antimicrobial drugs complicates the treatment of enterococcal infections. Acquired resistance to high concentrations of ampicillin, aminoglycoside, and glycopeptides antibiotics, specifically vancomycin, has exacerbated this problem. Highest prevalence of resistance was observed against penicillin (85%) followed by ciprofloxacin (67%) and vancomycin (65%). Of the 100 isolates, 77 % were multiple drug resistant. Both *E. faecalis* and *E. faecium* exhibited >57% resistance for ciprofloxacin in this study. High level of ciprofloxacin resistance has been reported by Anbumani et al., and Shah et al., where ciprofloxacin resistance accounted for 58% and 62% respectively. The isolates in this study showed 10% sensitivity to doxycycline.^[11]

High level gentamicin resistance (HLGR) was observed in 66% of the enterococcal isolates in our study. This finding correlates with the Anbumani et al., Shah et al., and Fernandez et al studies where HLGR was 56%, 53% and 53% respectively. Studies conducted in New Delhi and Mumbai have reported HLGR prevalence to be as high as 70 and 100 percent, respectively.^[12]

In this study, the occurrence of HLGR among the enterococcal isolates had no significant difference seen between *E. faecalis* and *E. faecium* isolates. However Mendiratta et al., have reported greater resistance to HLG among *E. faecium* as compared to *E. faecalis* isolates.^[10]

In this study the VRE isolation was 7%. Studies from Indore and Nagpur reported 14.29 and 11.38% VRE, respectively (Chitin et al., Rahangdale et al.,). In India, the prevalence of VRE has been reported to be between 0 and 30 %. In our study, among the VRE 3 were *E. faecalis* 1 was *E. faecium*. This is similar to the finding by Agarwal et al., who found vancomycin resistance to be greater among *E. faecalis* isolates.^[11,12]

Of the 7 VRE isolates 4 VRE were found to be susceptible to high level gentamicin. Hence, that infection could be treated with a combination of a high-level aminoglycoside and a lactam antibiotic. The presence of high-level gentamicin resistance and concurrent resistance to Penicillin or Ampicillin and vancomycin has been reported in some studies. An USA based study demonstrated that gentamicin resistance plasmid might cotransfer vancomycin resistance plasmids. Hence the detection of high-level gentamicin resistance along with vancomycin resistant enterococci represents a significant problem in this region.

On studying the susceptibility pattern of VRE isolates to supplemental drugs like linezolid, Quinupristin and chloramphenicol, all of the he VRE isolates were susceptible to Linezolid and Quinipristin (100% sensitivity). 55% of the isolates showed resistance to chloramphenicol. The study carried out by Gupta et al., from Chandigarh, India and MM Salem Behkit et al., from Iran have reported, 100% sensitivity of VRE isolates to linezolid which is similar to our study. Agarwal et al., has also reported 100% sensitivity to linezolid in their study. Perlada et al., from Australia also have reported 100% sensitivity to linezolid and 100 % sensitivity to Chloramphenicol. But in our study 55% of VRE isolates were resistant to Chloramphenicol.

In conclusion, Enterococci are emerging as an important pathogen causing variety of nosocomial infections and also cause community acquired infections contributing significantly to patient's morbidity and mortality.

The emergence of Vancomycin resistant Enterococci worsens the problem further because of the Multidrug resistance exhibited by these agents leaving fewer therapeutic options for the clinicians in treating the serious life threatening VRE infections.

CONCLUSION

The prevalence of VRE varies based on geographic location, antibiotic use of the subject population. This study emphasises the need for conducting frequent surveillance. Programmes for prompt identification of VRE in hospitals and community. This also highlights the need for implementation of stringent infection control measures like rational use of antibiotics especially restricting the use of Vancomycin to minimum, proper containment and effective treatment of VRE infections, strict hand washing practices, education of the healthcare workers and other personnel involved in the patient management. These measures are to be strictly followed to bring down the mortality and morbidity associated with these hospital acquired VRE infections.

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